DEVELOPMENT OF NEAR INFRARED REFLECTANCE SPECTROSCOPY (NIRS) CALIBRATIONS OF MAIZE KERNEL PHOSPHORUS FOR THE IDENTIFICATION OF USEFUL BREEDING MATERIAL

A Thesis

by

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ABSTRACT

Analysis of grain samples for nutrient composition is useful for breeding crops with improved nutritional, industrial or agronomic value. Wet chemistry analysis for composition components can be costly and laborious; therefore, a need exists for plant breeders to rapidly screen breeding material in a non-destructive manner. This study examined the application of near infrared reflectance spectroscopy (NIRS) calibrations to predict composition components, phosphorus in particular, in whole and ground maize (*Zea mays* L.) kernel samples using a specific Fourier Transformed NIRS (FT-NIRS) machine. Phosphorus, although an essential plant nutrient, has the potential to be an environmental pollutant. Therefore as maize production continues to increase globally, plant breeders need the ability to rapidly analyze nutrient profiles in breeding stock in order to select lines for advancement to achieve quality and environmental goals.

An initial experiment was conducted to identify the optimal NIRS scanning procedure for the FT-NIRS, specifically a Thermo-Fisher Antaris II. We determined that for maize sample analysis, the optimal number of scans for consistency, accuracy, and analysis time was 128 for whole kernel, 64 for 1 mm fineness, and 96 for 2 mm fineness. Calibration development of NIRS was facilitated through a diverse sample set in which composition components (crude protein, phosphorus, fat, and starch) were quantified by wet chemistry analysis at a commercial laboratory. The addition of other components gave a baseline for comparison with the phosphorus calibration. We found that whole kernel maize samples (performance index, an independent measure, [PI] =60, r=0.94) were nearly as predictive as ground maize kernel samples (PI=63, r=0.88) for

phosphorus. Several thousand samples that were in the TAMU Maize Breeding and Quantitative Genetics program's NIRS database, were analyzed to find genotypes with high and low phosphorus levels ranging from 0.27% to 0.43%. Selected genotypes were planted in an experimental test to validate predictions on their phosphorus levels and the effects that controlled pollinations play. These results indicated that phosphorus levels could be characterized categorically from both whole kernel and UDY calibrations, although some inconsistencies with expectations were found. Consequently, FT-NIRS could easily be integrated into a breeding program for rapid selection of genotype specific composition profiles.

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NOMENCLATURE

FT-NIRS	Fourier-Transformed Near Infrared Reflectance Spectroscopy
LPA	Low Phytic Acid
P	Phosphorus
PI	Performance Index
NIR	Near Infrared Reflectance
NIRS	Near Infrared Reflectance Spectroscopy
TAMU	Texas A&M University

TABLE OF CONTENTS

Page

LIST OF FIGURES

Page

LIST OF TABLES

Page

CHAPTER I

INTRODUCTION

Maize (*Zea mays L.*) is an important agronomic crop grown worldwide for human consumption, livestock feed and biofuel. The United States leads global production with approximately 361 million tons produced annually (FAO, 2015). Global production has increased within a 10-year period from 645 million tons annually in 2003 to 1.0 billion tons in 2014 (FAO, 2015).

As maize production continues to increase globally, breeders need the ability to rapidly analyze nutrient profiles in breeding stock in order to select lines for advancement to achieve compositional quality and environmental goals. Through breeding programs, maize composition can be altered in a variety of ways to develop lines that better suit environmental as well as human needs. Since maize grain is sold by weight and not by composition, levels of nutrients and minerals can vary significantly. Through the development of a cost effective screening method, breeders could potentially have the ability to select for desired nutrient profiles for specific end use of maize grain. In feedstuffs, low phosphorus levels are ideal in order to reduce nutrient enrichment of water bodies in the United States. Ideally, plant breeders would be able to select and develop maize lines that not only store less phosphorus, but also could thrive on less phosphorus fertilizer applied without negatively affecting development or yield.

Phosphorus levels in maize grain are generally tested through the use of the analytical chemistry methods (hereon referred to as wet chemistry analysis), which can be time consuming and cost prohibitive for livestock feeds, as well as agronomy

research and breeding programs (Balthrop et al., 2011). In addition, the destruction of breeding material through wet chemistry is necessary which can often put breeders in a situation of low reserves for future planting of seed in field trials.

The goal of this research was primarily to determine the effectiveness of Near Infrared Reflectance Spectroscopy (NIRS), specifically utilizing a Fourier Transformed NIRS (FT-NIRS) machine, as a viable screening method for quantitative analysis of phosphorus in maize grain, which has not previously been reported to our knowledge. NIRS analysis does not require the destruction of breeding material, is low cost and allows for rapid decision making. While NIRS has long been used in agriculture in general and maize in particular, there have been few studies on any composition phenotypes using FT-NIRS, which has a number of advantages to the more routinely used scanning monochrometer machines (e.g. FOSS). In addition to investigating phosphorus in maize, crude protein, fat and starch levels were also investigated to give this study a baseline for comparison with the phosphorus calibrations developed.

This thesis includes a best practices study that determined the ideal scanning procedures and identified sources of variation in the scanning process. In addition, this study described the development of NIRS calibrations and the accuracy of whole kernel calibrations in comparison to ground kernel calibrations. Calibrations were applied to thousands of previously scanned samples to identify high and low phosphorus lines. These lines were then planted in a winter nursery and crossed in various ways to validate phosphorus levels and evaluate the ability of the developed calibrations to rank phosphorus levels as high or low.

CHAPTER II

REVIEW OF THE LITERATURE

Phosphorus, in addition to nitrogen and potassium, is one of the three major essential nutrients necessary for growth and development in plants. Phosphorus is a required component for the formation of adenosine triphosphate (ATP), which is a major catalyst for many plant biochemical processes. Phosphorus is crucial to tissue development and DNA formation and aids the plant in nutrient uptake from the soil. Phosphorus deficiency symptoms include stunted growth, delayed maturity, poor seed quality, and reduced yield. Therefore phosphorus can only be reduced in the seed to a certain level until it negatively impacts plant growth and yield.

Despite the overwhelming importance of phosphorus to plant health, research has shown that plant seeds can also accumulate more phosphorus than what is required to support cellular functions (Raboy, 1997). Approximately 65-80% of a mature maize seed's phosphorus content is in the form of myo-Inositol (1,2,3,4,5,6) hexakisphophate ($InsP_6$ or phytate) (Raboy et al., 2000), a salt of phytate. Nearly 80% of this phytate is stored in the seed's embryo with the remaining amount being found in the aleurone layer (Raboy et al., 2001). Although the majority of total P in a seed is phytate, it seems reasonable to evaluate maize lines for low total P in contrast to low phytate specifically, which has been the focus of other studies. Total P and phytate have been shown to be positively correlated ($r>0.90$) so naturally a reduction in total P would lead to a decrease in phytate (Raboy, 1997).

Phytate and its various forms are of vital importance in eukaryotic cells for a variety of developmental and signaling processes within the cytoplasm and plastids. Processes include signal transduction involving transient calcium flux and the storage of various minerals (Raboy, 2009). InsP6 has also been shown to contribute to the physiological response of guard cells to abscisic acid (Lemtiri-Chlieh et al., 2000).

Despite the great importance P plays in crop growth and development, effective P fertilization presents some practical challenges to producers: 1) P may be tightly bound to clays and remain unavailable to the plants, 2) P is less mobile in the soil than other nutrients, and 3) P is becoming more expensive as cheap sources, such as rock phosphate, are rapidly disappearing. It has been estimated that between the years 2033-2100, high quality phosphorus reserves may be exhausted (Cordell and Neset, 2014).

Unlike nitrogen, which can be fixed by plants through microbial symbiosis, phosphorus is a resource that can only be made available through mining processes, which are controlled by six countries in the world (Cordell and Neset, 2014). Increasing P use efficiency is critical to sustain crop productivity under limited P supply. Consequently, maize breeders need the ability to select cultivars that can uptake and function with lower amounts of total P.

While there is a critical need to increase P use efficiency for sustaining crop yields, the off-target movement of phosphorus is the subject of much concern in the United States as the eutrophication of freshwater in the United States continues to occur. Eutrophication occurs as a result of excess nutritional supply in water bodies, leading to algal bloom and associated negative impacts; excess P leaching from agricultural

production fields is known to greatly contribute to eutrophication (Daniel et al., 1998). Sources of excess P include animal manure, plant residues and commercial fertilizer.

Monogastric animals such as swine and poultry are unable to digest phytic acid because they lack the enzyme phytase (Cromwell and Coffey, 1991). Consequently, their manure is rich in P and when applied to agricultural fields in large amounts, it contributes to freshwater eutrophication through run-off. With an increase in confined feeding operations, phosphorus can become increasingly concentrated on the landscape and changes from a valuable nutrient to a dangerous pollutant.

The devastating impact of excessive phosphorus on United States water bodies was first identified in the 1970's (Ryden et al., 1973) with sources of excessive P enrichment classified as either point source or nonpoint source (NPS) pollution. Phosphorus enrichment from animal manure and crop residues are classified as nonpoint source pollution because the nutrients or contaminants are picked up by rainfall or snowmelt that can either cause water run-off or leaching (EPA, 2016). This contamination has increased with the feeding of grain to animals that cannot digest it, and the concentration of feeding, and hence manure to localized regions (Duda and Finan, 1983). Because maize is the most widely fed grain in the U.S. (USDA-ERS, 2016), it is a logical place to begin to reduce P in the feed which should reduce P in animal manure. We expect that P in the grain can be reduced by growing in low P environments including by reducing P fertilizer (which would reduce yield and is not practical), or by identifying maize germplasm that accumulates less P in the grain that

maintains yield. An ancillary benefit of identifying maize germplasm that accumulates less P in the grain is that it may also need less P fertilizer.

Phosphorus, in the form of phytic acid, acts as an anti-nutrient within the human body by binding micronutrients such as zinc and iron, thus rendering vital nutrients unavailable for use. Those most affected would be people in underdeveloped nations whose diets consists of cereal grains that are not fortified to provide missing micronutrients. Consequently, the ability to produce maize lines that have less phytic acid could help those in underdeveloped regions to have access to grain with higher levels of micronutrients. Research has, so far, primarily been conducted with low phytic acid maize mutant lines to see if a reduction in phytic acid levels would increase the availability of micronutrients. A 2005 study by Lin et al., showed that low phytic acid lines could increase iron availability by 1/3 in the grain compared to wild type maize lines.

Low Phosphorus Maize

Several approaches have been explored in the scientific community to mitigate the effects of high phosphorus in maize grain. Work has been done with low phytic acid mutants and with traditional phenotypic selection (Raboy, 2009). As with most strategies, there are caveats that can impede adoption of new technology or ideas. The development of Low Phytic Acid (LPA) maize populations was one past approach in the maize-phosphorus debacle. Mutations of maize were isolated to create LPA mutant lines that have a 30-90% reduction in phytate concentration (Raboy, 2009). Additionally, these mutant lines had an increase in iron (Fe) bioavailability (Mendoza et al., 1998) and

improved P utilization in hogs (Hill et al., 2009). Despite the positive discoveries, researchers found that these mutant lines had poor germination and low yields, both major factors in the lack of adoption by farmers (Raboy, 2009).

To our knowledge, LPA maize varieties have not been studied in regards to stress tolerance or pest and disease resistance. This is one advantage to screening maize lines developed through traditional breeding methods where plant performance is typically already known. Additionally, it is unknown how an LPA mutant would affect phenotypes in the genetic backgrounds of tropical maize varieties grown in Africa or Central and South America.

Recurrent selection is another approach that has been evaluated as a means for altering phytic acid levels, in order to increase iron bioavailability in maize. In a study by Beavers et al. (2015), two synthetic maize populations were utilized: BS11, a temperate Corn Belt line, and BS31, a tropical line. Individual ears were selected from both populations and screened for relative phytate levels. The Wade method, a colorimetric assay was used to determine phytate levels, which could not ascribe real values. Five of the highest phytate ears and five of the lowest phytate ears were selected from each population and bulked to create four lines. Plants from these populations were crossed in a chain-sib mating design. Screening for phytate levels occurred for each cycle of breeding and the ears with the highest and lowest phytate levels were planted to begin the next selection cycle. Analysis of these high phytic acid (HPA) and low phytic acid (LPA) populations originating from the BS11 population showed significant differences ($P < 0.001$) in phytic acid concentrations between each other and compared

to LPA mutant lines. However for the BS31 population, the samples were only significantly ($P < 0.05$) lower in extractable P from the LPA mutants. Phytate was only assessed through the use of relative rank, therefore, we are uncertain of the quantitative P values in the study. The study also evaluated genotype by environment (G X E) effects on extractable P and phytic acid concentrations. G X E effects were not significant for phytate however they were significant for extractable P. Although there was some reduction in phytic acid levels using recurrent selection, the number of samples needing wet chemistry analysis may be cost prohibitive for a recurrent selection approach to be economical.

Available Screening and Analytical Methods

Near infrared reflectance spectroscopy (NIRS) is an analytical tool, capable of providing spectral data useful to identify and estimate the composition of various solids, liquids and gases. NIRS can rapidly analyze samples for both quantitative and qualitative measurements and is an alternative method to traditional wet chemistry analytical methods and more modern methods such as high performance liquid chromatography (HPLC). NIRS has major advantages over wet chemistry of not requiring any consumables for making measurements, being near instantaneous in predictions, and allowing non-destructive sampling. Wet chemical analysis for components such as P typically would require destruction of the sample by grinding and chemical analysis, which can be quite time consuming. NIRS can be used on both ground kernel and whole kernel (non-destructive) samples for some components, but it is currently unknown to

our knowledge if NIRS will work for predicting P on either whole kernel or ground maize grain samples.

The NIRS method relies on near infrared light in the range of 780-2500 nm in the electromagnetic spectrum which penetrates through agricultural products, such as maize, to a varying depth of 1-2 mm (Manley et al., 2009) where the light is either absorbed or reflected (Batten, 2008). These spectral values can then be converted to predicted values based on existing wet chemistry-derived calibrations (as will be described later). NIRS technology works by recording the rotational, vibration or electronic energy caused by photon energy within a specific molecule. Most of the molecular vibrations detected by NIR spectroscopy are caused by hydrogen bonds to oxygen, nitrogen or carbon atoms but bonds to other compounds may also be detectible (Shenk et al., 2001) (Figure 1).

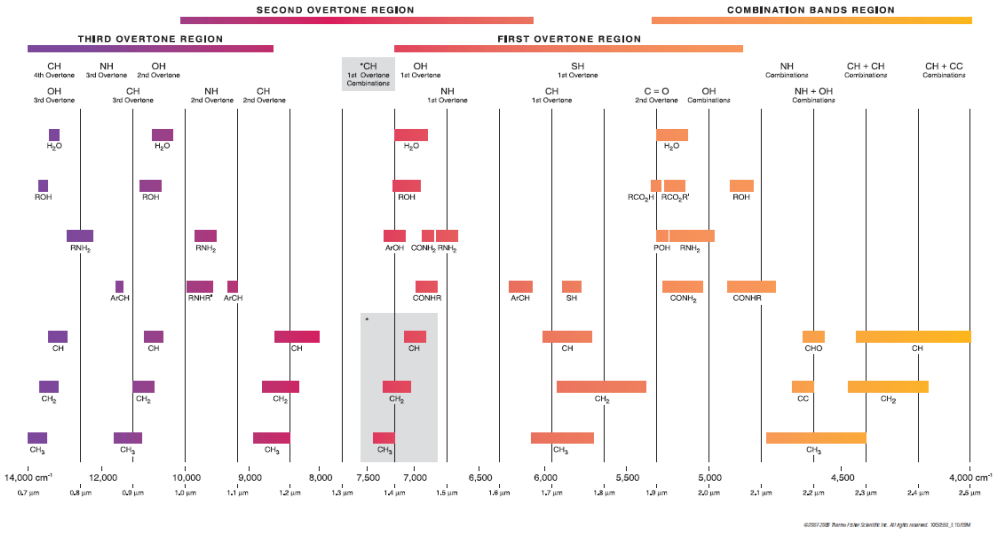


Figure 1. Table of NIR absorption bands (Thermo Fisher Scientific Inc. 2007 XX50550_E 11/07M)

Each compound has a unique spectrum and the height of the spectrum peaks are directly related to the level of different materials present in the sample.

Herschel first discovered the near infrared region in the 1800's when he was able to separate the electro-magnetic spectrum with a prism. It was observed that the temperature increased at and beyond the red portion of the spectra, which is now known as the infrared region. Even though a variety of experiments were conducted in the 1920's with NIR, it wasn't until the 1960's that NIR spectroscopy became a more common analytical procedure due to promotion by Karl Norris of the United States Department of Agriculture (USDA) (Shenk et al., 2001). Norris and his colleagues found that the diffuse reflectance bands could be used for rapid analysis of agricultural grains, in particular analyzing protein and fat in soybeans. NIR spectroscopy development continued into the 1980's with researchers finding uses for NIRS technology in testing forage quality. Portable NIRS instruments were subsequently developed. Today NIRS technology is popular in the natural resources sector (Reich, 2005) especially for soil analysis. Fourier-Transformed NIRS (FT-NIRS), a specific method of NIRS, has mainly been used in the pharmaceutical industry for analysis of pure compounds and has a number of advantages over traditional NIRS instruments, however it has only recently been used for agricultural products.

FT-NIR technology works through the use of an interferometer that has all the NIR frequencies encoded into it. A halogen light source produces the NIR energy that passes through the interferometer and is then either reflected off of or absorbed by the

sample. The signal is then sent back to the interferometer where the unique frequencies are digitized and sent to the computer where the spectrum is produced.

FT-NIRS technology has advantages over the more commonly used Foss monochrometer scanning machine, a dispersive instrument. The primary advantage of FT-NIRS is that they are mechanically simpler than dispersive instruments. The only moving part within the instrument is the mirror within the interferometer. When using dispersive instruments, gratings and filters are constantly moving to generate a spectrum. A decrease in mobile parts equates to an increase in machine robustness and reliability with few chances of mechanical breakdowns.

As previously stated, all NIR frequencies are encoded into the FT-NIRS machine's interferometer. In dispersive instruments, the frequencies are separated at 50 cm^{-1} apart (Thermo Fisher, 2015) and if the spectral information can't be measured at this resolution, then a slit mechanism is used to obtain sample readings. The caveat with a slit mechanism is that considerable energy loss occurs and consequently a loss of precision. FT-NIRS machines separate NIR frequencies at 8 cm^{-1} thus providing a higher resolution reading (Thermo Fisher, 2015). Since higher resolution scans are produced, less samples may be required to build accurate calibrations.

FT-NIRS also has an advantage known as the Connes Advantage since it utilizes an internal reference laser. The laser provides the machine with accuracy and precision of greater than 0.1 cm^{-1}. Dispersive machines again utilize prisms or grates which can cause inaccuracies between scans and peak position errors. This can lead to a sensitivity to vibration or bumping a dispersive machine, that an FT-NIRS does not have.

Consequently, reference materials must be utilized for calibration thus introducing the problem of operator error and more difficult calibration development.

NIRS technology has been utilized for analyzing various components in maize grain and forage. However, to our knowledge, no studies have been conducted to investigate the possibility of using NIRS to rapidly analyze total phosphorus in maize grain for screening breeding material and few have looked at using FT-NIRS to evaluate maize grain for compounds including standard crude protein, oil and starch estimation. Although NIRS has been used routinely for major grain compounds like starch, oil and protein, NIRS has more recently been evaluated for its use in quantifying a variety of micronutrients and antioxidants in maize. NIRS has been investigated for its use in quantitative analysis of iron, zinc and pro-vitamin A carotenoids in maize for improved varieties to combat micronutrient deficiencies in underdeveloped nations (Ortiz-Monasterio et al., 2007). A 2014 study by Meng et al. (2015) demonstrated that NIRS, and FT-NIRS in particular, could successfully estimate phenolic content in both whole and ground maize samples. In 2004, a study by Brenna and Berardo showed that the quantification of carotenoid levels in maize through NIRS was as effective as the more commonly used high-performance liquid chromatography (HPLC) with samples analyzed in a matter of minutes and only needing grinding.

NIRS technology has also been evaluated for its integration into breeding programs to determine tryptophan and lysine levels in quality protein maize (QPM) (Rosales et al., 2011). Researchers found that for 266 lines from five breeding populations, the coefficients of determination were 0.94, 0.76, and 0.80 for protein

content, tryptophan and lysine, respectively. Since tryptophan and lysine levels have to be routinely checked in QPM breeding programs, NIRS proved to be an efficient and economical alternative to more frequently used wet chemistry analysis methods.

NIRS technology has previously been successfully applied to analyze nutrient profiles in other agricultural crops as well. Fatty and amino acid levels in soybeans were quantified using NIRS (Pazdernik et al., 1997). Additionally, it was utilized to determine polymerized triacylglyceride in vegetable oils (Kuligowski et al., 2012). A 2013 study by Ferreira et al. utilized FT-NIRS, specifically that was used in this study as well, to estimate Brazilian soybean parameters including protein, moisture, ash, lipids, and carbohydrates. Qualitative assessments were made to differentiate between glyphosate resistant genetically modified (GM) soybean and non-GM soybean (Lee and Choung, 2011).

In this thesis, three separate but related activities were undertaken, a best management practices (BMP) study to determine the number of scans that should be used, the development of both whole kernel and UDY NIRS calibrations and subsequently, genotypes that were predicted through NIRS to have extreme high and low P levels were validated in a field study.

CHAPTER III

BEST PRACTICES STUDY

Introduction

FT-NIRS in general and the Thermo-Fischer Antaris II FT-NIR analyzer in particular have primarily been used in industries where mostly pure compounds would be investigated. It has been unclear what scanning procedures are needed for heterogenous agricultural samples. In order to develop the most accurate calibrations, the optimal scanning procedure for maize (*Zea mays* L.) was investigated. We specifically used measures that investigated repeatability and accuracy through identifying the percent of variation from multiple sources: scan replication, cup fill, the number of scans and genotype. The experiment was conducted on whole and ground (2-mm and 1-mm grind) maize samples. Our objective was to find the optimal number of scans for each particular grind and whole kernel maize. The ideal outcome would be the ability to utilize whole kernel samples for nutritional analysis and be able to obtain repeatable and accurate results without having to grind maize samples.

Breeding programs quite often are in need of obtaining nutritional composition to evaluate lines for advancement. At the TAMU- Maize Breeding Program, we routinely scan large numbers of samples into the FT-NIRS system that have unknown composition, with which we want to properly estimate component levels. The hypothesis for this best practices study is that as the number of scans per sample are increased, a decrease in the percent variation from cup fill and scan replication and an increase in variation from genotype will be observed. This inverse relationship should be

particularly evident in whole kernel samples that tend to have a greater variation due to irregular orientation of the kernels within the scanning cup. Ground samples should have higher percent variation from genotype and lower percent due to the remaining and unexplained factors even at low scan numbers due to the homogenization of the sample from grinding.

Methods and Materials

Sample preparation

The best practices study utilized whole and ground samples. Approximately 175 grams of each sample were ground to 2mm fineness using a Polymix PX-MFC 90 D mill (Kinematica Ag, Eschbach, Germany) (Figure 2). A Cyclone sample mill (UDY Corporation, Fort Collins, Colorado) was then used to grind the samples to 1 mm fineness (Figure 2).

Figure 2. Picture of prepared maize samples. L to R: Whole kernel maize, 2 mm fineness maize, and 1 mm fineness maize.

Near infrared reflectance spectroscopy for whole and ground kernels

Samples were scanned for spectral reflectance values using a Thermo-Fischer Antaris II FT-NIR (Thermo-Fischer Scientific, USA) analyzer. Reflectance measurements were taken by using a rotating cup that holds approximately 175g of maize over the instrument's integrating sphere module. Approximately, 3000 points across the spectrum, every 4 wave numbers, were collected for each sample scanned at a spectral range between 10,000 to 4,000 cm^{-1}.

Two diverse whole kernel maize samples were utilized including a yellow inbred (PB80) and colored hybrid (CS12-OTH-145) (((VS402 (hybrid blue)) X ((Lfy2361-B/Tx114 (B73w)–B Dark Blue-B) Tx114/Lfy2304-B-B-B-1-3-B-B-B-3-B-B)-1#/ (PHV63 (White) PI601500))/PHV63-B7. A sub-sample taken from each original sample was analyzed at five scan levels (averaging 16, 32, 64, 96, and 128 scans per sample). There were three technical replicate scans of each cup fill made at each scan level. In addition, there were a total of three separate cup fills for each sample. For each cup fill, the sample was poured out of the cup, remixed with the full sample and poured back into the cup. The above procedure resulted in a total of 135 observations across all replications of each scan level. The samples were ground to the 2 mm fineness and the scanning procedure was repeated. The scanning procedure was again repeated after the samples were then ground to 1 mm fineness. Whole kernel composition values were predicted based on the Whole-3-Cp and Whole-3-PFS calibrations; UDY ground maize samples were predicted based on UDY-2-CpP, UDY-2-F and UDY-2-S calibrations developed in Chapter IV.

Statistical analysis

The predicted values were exported and analyzed using JMP® software (Version 12.1, SAS Institute, Cary, NC). Sources of variation were identified whether they occurred from the sample, the technical replicate, the cup refill, or the grind. To show how the spectrum variation carried over into actual prediction variance, four composition predictions for multiple components (protein, starch, fat/oil, phosphorus) were made within TQ analyst (Thermo Scientific). The ideal number of scans were determined based on the number of scans that had the most variation from genotype and the least amount of error variation from the cup fill or technical replicate. The statistical model was run with genotype, cup fill, and replication as random effects. Genotype was nested in cup fill and cup fill and genotype were nested in replication.

Each component was predicted within TQ Analyst using a calibration with a specific combination of pre-processing factors determined based on early calibration development. Consequently, at the time this research was conducted, there were two calibrations for whole kernel samples, each one best predicting one specific component (crude protein, phosphorus, etc.). However, all four components prediction by each specific calibration were retained for comparisons. Consequently, there are two sets of prediction values for each component at the whole kernel level. For both 2mm and UDY (1 mm) grind levels, there were three sets of prediction values for each component. The predicted values utilized for each component were from the calibration within the grind types that was designated to be used for predicting that component.

Results and Discussion

For nearly every trait, scan, and grind combination, significant differences were observed between the two genotypes (Table 1 below). The best number of scans was selected based on maximizing the variation due to genotype, while minimizing the variation from cup fill and rep (i.e. error). Whole kernel samples tended to have as much variation from cup fill and replication, likely due to the orientation of the individual kernels in the sample cup. Samples showed less variation from cup fill when they were ground, likely due to the homogenization of the sample.

The suggested optimal number of scans was determined to be 128 for whole kernel, 96 for 2 mm fineness, and 64 for 1 mm fineness. For half of the components, including phosphorus and fat, scanning at a level of 128 scans for whole kernel samples, showed the most variation due to genotype and the least variation from cup fill and replication. For whole kernel crude protein specifically, 64 scans had the most variation from genotype, 33.5% compared to 13.1% for 128 scans, which could not be explained. Whole kernel starch estimates showed the least variation from rep and cup fill at a scan level of 64, and slightly more genotypic variation (80.3%) compared to 70.5% at 128 scans. Whole kernel phosphorus showed a drastic difference in genotype variation when comparing 36.0% at 96 scans to 73.8% at 128 scans.

When evaluating ground 2mm samples, crude protein and fat both exhibited the same trend with 128 scans showing the most variation from genotype. However the difference between the 96 and 128 scan levels for genotype variation was a small percentage (<2%) so we felt that the tradeoff for saving time in the scanning procedure

was worth the difference in selecting 96 scans. Phosphorus and starch both had the highest genotypic variation and the lowest variation from cup fill and replication using 96 scans. Phosphorus, in particular, exhibited a 13.6% decrease in genotype variation when increasing from 96 to 128 scans and a 19.1% decrease when decreasing from 96 to 64 scans.

For crude protein, fat, and starch in UDY samples, 64 scans was selected as the ideal number of scans due to the high percentage of genotype variation found at this level. Phosphorus showed the highest genotype variation at 128 scans (87.4%) compared to the selected level of 64 scans (72.3%).

Table 1. Percent variance component estimates based on number of scans, cup fill, and replication for individual composition components A) crude protein, B) phosphorus, C) fat, and D) starch. Bold numbers indicate the best scan level for each component and grind level.

A) Crude protein	**Whole kernel**					**Ground maize (2mm)**					**Ground maize (UDY-1mm)**				
Scan level	16	32	***64***	96	128	16	32	64	96	***128***	16	32	***64***	96	128
Genotype	0.0	0.4	***33.5***	0.0	13.1	90.4	95.0	95.2	95.2	***96.6***	98.8	99.7	***99.8***	99.6	99.4
Cup fill (genotype)	0.0	34.0	***32.4***	49.6	32.2	6.4	2.4	3.7	4.4	***3.3***	0.3	0.0	***0.1***	0.0	0.5
Rep (cup fill, genotype)	100.0	65.6	***34.1***	50.4	54.7	3.2	2.7	1.1	0.4	***0.1***	0.9	0.3	***0.1***	0.4	0.0
Total	100.0	100.0	***100.0***	100.0	100.0	100.0	100.0	100.0	100.0	***100.0***	100.0	100.0	***100.0***	100.0	100.0

B) Phosphorus	**Whole kernel**					**Ground maize (2mm)**					**Ground maize (UDY-1mm)**				
Scan level	16	32	64	96	***128***	16	32	64	***96***	128	16	32	64	96	***128***
Genotype	0.0	*54.8*	53.0	36.0	***73.8***	*56.1*	17.4	11.0	***30.1***	16.5	51.2	73.2	72.3	81.9	***87.4***
Cup fill (genotype)	0.0	*0.0*	22.5	34.1	***9.0***	16.7	23.1	*11.5*	***53.4***	66.5	32.0	24.8	25.4	12.2	***11.9***
Rep (cup fill, genotype)	100.0	45.2	*24.6*	29.9	***17.2***	27.3	59.5	77.5	***16.6***	17.1	16.8	1.9	2.4	5.8	***0.7***
Total	100.0	100.0	100.0	100.0	***100.0***	100.0	100.0	100.0	***100.0***	100.0	100.0	100.0	100.0	100.0	***100.0***

C) Fat	**Whole kernel**					**Ground maize (2mm)**					**Ground maize (UDY-1mm)**				
Scan level	16	32	64	96	***128***	16	32	64	96	***128***	16	32	***64***	96	128
Genotype	9.7	77.3	68.9	76.6	***81.1***	83.8	*96.5*	94.0	95.8	***97.4***	95.0	95.7	***98.3***	97.0	97.6
Cup fill (genotype)	0.0	0.2	14.9	*7.4*	***11.8***	3.7	*1.4*	4.6	3.9	***2.5***	3.0	2.8	***1.4***	2.1	2.1
Rep (cup fill, genotype)	90.3	22.5	16.2	16.0	***7.1***	12.5	2.1	1.4	0.3	***0.1***	2.1	1.5	***0.3***	1.0	*0.2*
Total	100.0	100.0	100.0	100.0	***100.0***	100.0	100.0	100.0	100.0	***100.0***	100.0	100.0	***100.0***	100.0	100.0

D) Starch	**Whole kernel**					**Ground maize (2mm)**					**Ground maize (UDY-1mm)**				
Scan level	16	32	***64***	96	128	16	32	64	***96***	128	16	32	***64***	96	128
Genotype	0.0	23.3	***80.3***	64.6	70.5	0.0	17.1	35.1	***89.0***	81.9	40.5	54.8	***59.9***	56.8	25.5
Cup fill (genotype)	0.0	25.9	***12.4***	1.4	11.5	0.0	42.2	24.3	***7.6***	14.9	50.9	41.3	***39.7***	31.6	73.3

Table 1 continued.

D) Starch	Whole kernel					Ground maize (2mm)					Ground maize (UDY-1mm)				
Rep (cup fill, genotype)	100.0	50.7	***7.3***	34.0	18.0	100.0	40.8	40.6	***3.4***	3.2	8.5	3.9	***0.4***	11.6	1.3
Total	100.0	100.0	***100.0***	100.0	100.0	100.0	100.0	100.0	***100.0***	100.0	100.0	100.0	***100.0***	100.0	100.0

CHAPTER IV

DEVELOPMENT OF WHOLE KERNEL AND UDY NIRS CALIBRATIONS

Introduction

To be able to predict unknown samples a calibration first needs to be built. An ideal calibration will be able to predict nutritional components in samples with diverse genotypes and grown in varied field environments. The objectives in this study were to 1) develop basic calibrations using an iterative procedure; 2) evaluate the performance of these calibrations with known calibration and unknown validation samples; and 3) use these calibrations to select additional samples for the calibration. We hypothesized that a robust and useful calibration could be achieved through the incorporation of diverse samples as standards. The UDY calibration was likely to be the most accurate due to homogenization of the sample, however, we believed that a whole kernel calibration should at least have the capability of ranking low to high values of composition components, making it ideal for breeding line evaluation and advancement.

Methods and Materials

Grain samples used in calibration development

For calibration development the procedure was iterative. First, diverse samples scanned in from many various experiments to be predicted from NIRS. A fixed number of samples (depending on resources) representative of the extremes in sample diversity in the set were selected for wet chemistry analysis. Next, these were then used to build a NIRS calibration. The best calibration was then used to predict all maize grain samples that had been scanned in up to that point. In subsequent iterations, samples predicted as

outliers for each trait (highest and lowest) were selected for additional wet chemistry; the number of samples depended on the financial resources. Finally, a new calibration that included all samples for wet chemistry was built. It should be noted that outliers for any one composition trait, tended to be more "normal" for other composition traits, so "normal" samples did not need to be intentionally selected. This iterative procedure was performed multiple times per year with new samples.

At the time of this thesis, a total of 225 diverse maize grain samples had been included for whole kernel calibration development and 270 samples were included for the UDY grind calibration development. Composition values were obtained from wet chemistry analysis by Ward laboratories as described later. These maize samples were generated from field experiments conducted by the Texas A&M Quantitative Genetics and Maize Breeding program (College Station, TX) between the years 2006-2015 and included very diverse experiments, germplasm, and locations; many of the samples used have been reported in other studies (Farfan et al. 2015, Meng et al. 2015, Mahan et al. 2013, Mahan et al. 2014, and Wahl et al. 2016). In addition, samples were obtained from other maize breeding programs across the United States to help develop a more robust calibration targeted at the maize ATLAS project (http://maizeatlas.org). The samples included hybrid and inbred material from temperate and sub-tropical/tropical growing environments.

Sample preparation

After whole kernel samples were scanned using a Thermo-Fischer Antaris II FT-NIR analyzer(Thermo-Fischer Scientific, USA), approximately 175 grams of each

sample were ground to 2mm fineness using a Polymix PX-MFC 90 D mill (Kinematica Ag, Eschbach, Germany) and then further ground to 1mm fineness using a Cyclone sample mill (UDY Corporation, Fort Collins, Colorado) and scanned again.

Near infrared reflectance spectroscopy for whole and ground kernels

Reflectance measurements were taken by using a rotating cup on the Antaris II FT-NIR that held approximately 175g of whole kernel maize over the instrument's integrating sphere module. Whole kernel samples were run with 128 scans per sample and 64 scans per sample were used for 1mm fineness ground samples. Approximately, 3000 points across the spectrum, every 4 wave numbers, were collected for each sample scanned at a spectral range between 10,000 to 4000 cm^{-1}.

Nutrient profile from wet chemistry analysis

Samples were sent to Ward Laboratories (Kearney, NE) for wet chemistry analysis of crude protein, phosphorus, fat, starch, moisture, calcium and acid detergent fiber (ADF). Samples were reported on percent dry basis. All methods of wet chemistry analysis were obtained from www.wardlab.com (date accessed 9/1/2016).

Phosphorus levels were determined using the digestion method on an ICAP machine (information from www.wardlab.com). Individual maize subsamples (1-mm grind) were weighed prior to digestion and placed into digestion tubes. Approximately 3 mLs of hydrochloric acid (HCl) and 6 mLs of nitric acid (HNO_3) were dispensed into the samples. The sample vials were placed into a digestion block at 90 degrees Celsius for 105 minutes. 1 mL of 30% hydrogen peroxide (H_2O_2) was dispensed into the vials and the digestion block was turned up to 140 degrees. After 15 minutes, another 1 mL of

hydrogen peroxide was added to vials. The addition of hydrogen peroxide in 15 minute intervals was repeated two times. After samples were cooled, they were brought to a final volume of 50 mLs, mixed well and filtered. The samples were run on an ICAP machine according to standard operation protocol. Phosphorus levels are expressed as percent sample and were calculated using the following equation: % P = (Result from ICAP * 50) / Sample Weight.

Crude protein was determined according to the combustion method developed by Dumas and later modified by Sweeney (Padmore, 1990). Crude protein is a measure of the total nitrogen in a feed sample and includes true protein and non-protein nitrogen (NPN) such as urea. The method utilized a LECO FP-2000 Nitrogen Combustion Analyzer and thermal conductivity detector. Approximately 0.2 g of ground subsample (1-mm grind) was ignited at 1050 degrees celsius in the LECO FP-2000 analyzer in which elemental nitrogen was converted to N_2 and N_x. Combustion gas was passed through a copper catalyst to convert nitrous oxide to N_2 and remove carbon dioxide. Every 10^{th} sample was a calibration standard of EDTA. The percent nitrogen content was determined through thermal conductivity and multiplied by 6.25 to quantify crude protein values.

Starch was determined through the use of enzymatic determination. Approximately 0.3 g of ground subsample (1-mm grind) was put into a 100 mL flask along with 25 mL distilled water. The solution was mixed thoroughly and 10 mL 2N sodium hydroxide (NaOH) was added to the flask. After thorough mixing, the flask was heated on a hot plate and simmered for 20 minutes. 10 mL 2N hydrochloric acid (HCl)

was mixed into the flask and it was cooled to below 50 degrees C. 10 mL acetate buffer, 2.75 mL alpha amylase, and 2 mL amyloglucosidase were added to the flask sequentially and thoroughly mixed after each addition. After flasks were in a 45 degree C water bath for 70 minutes, 2 mL zinc sulfate solution and 1 mL 1N sodium hydroxide was added to stop hydrolysis and precipitate protein. Flasks were cooled to room temperature and brought to volume with phosphate buffer. The solution was mixed well and filtered. The liquid sample was added to a test tube and analyzed following standard protocol on the YSI 2700 analyzer. The percent starch was determined by the following equation:

$$\%\text{Total starch (dry basis)} = \left[\left(\frac{\text{dilution factor} \times \text{g/L glucose} \times 0.9}{1000}\right) / \left(\frac{\%\text{ dry matter}}{100}\right)\right] \times 100$$

The dilution factor and g/L glucose are generated by the YSI 2700.

Crude fat was determined by utilizing the ANKOM method. 1.0 g of subsample (1-mm grind) was dried in a convection oven for 3 hours at 105 degrees C. Samples were cooled and weighed again. Samples were evenly spaced in an extraction vessel and placed in the Ankom XT20 analyzer for 45 minutes. After the extraction cycle, samples were placed in the oven for 2 hours at 105 degrees C, cooled to room temperature and weighed. Crude fat was expressed as percent sample and calculated by the following equation: %Crude Fat= ((A-B)/C) x100; where A= weight of pre-dried sample, B= weight of extracted sample and C= weight of sample.

All wet chemistry samples were corrected for % moisture, also measured by Ward Labs (Ward Laboratories, Kearney, NE), were reported on a dry matter basis as % of the total sample and included in the NIRS calibration on a dry matter basis.

Calibration development

The calibration samples with known composition (crude protein, phosphorus, starch, fat, acid detergent fiber, and calcium) from wet chemistry analysis were used as standards. Initially, 112 samples were selected for wet chemistry based on the diversity of the spectra, a preliminary calibration was developed and then used to predict a set of high and low outliers for each composition trait (phosphorus, starch, fat, and crude protein)(Table 2). NIR predictions were analyzed using JMP software to identify outliers that fell outside of the normal distribution. We decided to look at all outliers regardless of a specific deviation from the mean. All outliers were then rescanned to confirm that they were indeed an outlier and not showing extreme nutrient levels due to a compromised NIR scan. Once this determination was made, the outlier samples were analyzed using wet chemistry methods. The nutrient profile results from these samples were combined with the original NIR nutrient predictions and a new calibration was developed. This process was repeated multiple times (at least once each year) to increase the number and diversity of calibration samples and improve the robustness of prediction. Within each calibration, approximately two-thirds of the samples were selected by the software as calibration standards and one-third were selected for validation standards. Additionally, the software selected samples to be ignored in the calibration. The most recent calibration has a total of 225 standards for whole kernel and 270 for UDY powder, thereby more than doubling the number of initial standards used (Table 2) in the original calibration. Over the course of calibration development, certain combinations of preprocessing methods resulted in more accurate predictions of

individual components. As a result, there could be a calibration best suited for predicting crude protein versus phosphorus. This will be discussed in more detail later in this chapter.

Table 2. Development history for whole kernel and UDY calibrations.

Calibration name	Predicts for:	Calibration model	Number of calibration standards	Number of validation standards	Ignores	Total calibration samples
Whole kernel						
Whole-1-Cp	Cp	PLS_Constant_D2_ND_3-2	112	0	0	112
Whole-1-P	P	PLS_Constant_D2_ND_3-1	112	0	0	112
Whole-1-F	Fat	PLS_Constant_D2_ND_3-5	112	0	0	112
Whole-1-S	Starch	PLS_Constant_D2_ND_3-3	112	0	0	112
Whole-2-Cp	Cp	PLS_Constant_D1_SG_7_1	112	35	6	153
Whole-2-PFS	P, fat and starch	PLS_Constant_D1_SG_11-5	112	35	6	153
Whole-3-Cp	Cp	PLS_Constant_D1_SG_7_1	123	35	6	164
Whole-3-PFS	P, fat and starch	PLS_Constant_D1_SG_11-5	123	35	6	164
Whole-4-Cp	Cp	PLS_Constant_D1_SG_7_1	144	50	31	225
Whole-4-PFS	P, fat and starch	PLS_Constant_D1_SG_11-1	161	55	9	225
Whole-5-CpPFS	Cp, P, fat and starch	PLS_Constant_D1_SG_3-6	165	49	11	225
UDY powder						
UDY-1-Cp	Cp	PLS_Constant_D1_ND_7-7	112	0	0	112
UDY-1-P	P	PLS_Constant_D2_ND_1-1	112	0	0	112
UDY-1-FS	Fat, starch	PLS_Constant_D1_ND_1-1	112	0	0	112
UDY-2-CpP	Cp and P	PLS_Constant_D1_SG_7-1	130	44	13	187
UDY-2-F	Fat	PLS_Constant_D1_SG_11-5	130	44	13	187
UDY-2-S	Starch	PLS_MSC_D1_ND_3-5	130	44	13	187
UDY-3-CpP	Cp and P	PLS_Constant_D1_SG_7-1	148	48	13	209
UDY-3-F	Fat	PLS_Constant_D1_SG_7-5	153	44	13	209
UDY-3-S	Starch	PLS_Constant_D1_ND_3-5	153	44	13	209
UDY-4-CpPFT	Cp, P, Fat and Starch	PLS_Constant_1D_SG_3-6	187	58	25	270

Data pre-processing and summary statistics

Raw spectral data was treated in TQ analyst software (Thermo-Fisher Scientific, United States) to reduce the amount of error that was not related to its physical or chemical information. 180 varying combinations of pre-processing techniques, smoothing methods and types of raw data were used to identify the optimal calibration model with the least error variation. Even though 180 combinations were observed, only the best calibration models were recorded. 54 models were recorded for whole maize (Appendixes F-I) and 16 for UDY grind calibrations (Appendixes J-M).

All combinations utilized the Partial Least Squares (PLS) method, a statistical approach based on the PLS algorithm which relates dependent variables to independent variables (Lorber et al., 1987). PLS utilizes multiple regions of the spectra for analysis and is capable of quantifying sample components when there is a complex correlation between concentration and absorbance. PLS was used to obtain the number of factors used in the prediction model. TQ software automatically determined the number of PLS factors based off of the prediction residual error sum of squares (PRESS). Models with a smaller number of factors were more desirable since a high number of factors could lead to an overfit model.

One of two pathways (pre-treatments), Multiplicative Signal Correction (MSC) or Constant, was utilized. MSC is able to compensate for differences in pathlength and can be used to normalize spectral data. The constant pathway uses one pathlength value to calculate the concentration of component amounts. This pathway is used under the assumption that all spectral values were collected at the same pathlength (which these

generally were). The calibration tested raw, first or second derivative functions. The data was collected at very high resolution so smoothing options of either the Norris Derivative or the Savitzky-Golay filters were explored. Norris Derivative can help to reduce the effect that random noise had on peaks in the spectra. However, it could only be used with the first or second derivative function and not the raw spectra. The degree to which data was smoothed was dependent upon the number of data points in each segment. The number of data points per segment could be set to any odd number from 1-25. As the number of data points increased, the amount of smoothing increased as well. The overall goal was to use the fewest data points to achieve an acceptable calibration result. In addition, the gap between segments was adjusted numerically from 1-12. As the size of the gap increased, there was a decrease in spectral resolution. A separate but similar filter, the Savitzky-Golay filter, determined the shape of the spectral curve by setting the polynomial order from a value between 2 and 6. More smoothing resulted from a higher polynomial order and less smoothing came from a lower polynomial order.

Data treatment techniques were evaluated by comparing several summary statistics including the correlation coefficient (r), performance index (PI) and root mean square error of calibration (RMSEC). For each pre-processing combination, the summary statistics were recorded so comparisons could be made and the best calibration model chosen. The correlation coefficient (r), described how strong of a relationship existed between two variables (Asuero et al., 2006). Root Mean Square Error of Calibration (RMSEC) refers to the residuals of the calibration data and measures the

goodness of fit between the data and the calibration. It is expressed numerically with a number closer to 0.00 indicating a low level of uncertainty. The Performance Index (PI) is unique to TQ Analyst software and is based off of the following equation: Performance Index (% difference) = (| actual-calculated |) / (expected range) X 100. The PI computes how the overall performance of a calibration looks when comparing actual values to predicted values and is expressed numerically with a number close to 100 being ideal. The number of PLS factors used in the calibration was also utilized in the evaluation process. All four factors were recorded and a prediction model was chosen based off of the combination that produced an r value closest to 1.00, RMSEC closest to 0.00, PI closest to 100, and the minimal number of factors used suggested by the software. Since no one calibration was the best for all of the criteria, some subjective judgement was used, placing the PI as the top criteria.

Results and Discussion

Phosphorus content based on wet chemistry

In order to develop the most robust calibration, samples that came from a variety of growing environments and experiments were utilized. Research has shown that NIRS is extremely accurate at predicting composition components when a homogenous set of calibration standards and samples were used for evaluation (Delwiche et al., 2006). However, this project sought to develop a calibration that was capable of analyzing any maize grain samples grown in any diverse environment.

The calibration sample set had a phosphorus range of 0.24-0.48% and 0.22-0.50% for the whole kernel and UDY calibrations, respectively for the wet chemistry

results (Table 3); the difference in whole kernel and UDY samples was due to a difference in the exact samples used. In the case of crude protein, a two-fold increase was observed as well, with a range of 6.9-15.9% for both the whole and UDY samples (Table 3). For fat, both whole and UDY had a range of 2.1%-6.7%. The values for starch ranged from 56.1%-76.4% and 55.5%-76.4% in whole and UDY respectively (Table 3).

Table 3. Mean, maximum, and minimum values for composition components in whole kernel and UDY grind calibration standards based on wet chemistry values (all values expressed as percent of the total sample weight and on percent dry basis).

Whole	**Crude protein %**	**Phosphorus %**	**Fat %**	**Starch %**
Min	6.9	0.24	2.1	56.1
Max	15.9	0.48	6.7	76.4
Mean	10.7	0.34	4.4	65.9
UDY				
Min	6.9	0.22	2.1	55.5
Max	15.9	0.50	6.7	76.4
Mean	10.8	0.34	4.3	65.8

Results of whole and ground kernel calibration

Both the whole and UDY ground kernel models utilized the same calibration building methodology with a difference in number of calibration samples and sample homogeneity. The best fit calibration model took several factors into consideration however a high PI and r and low RMSEC was not found in any model that could satisfy all the requirements. Among the 70 calibrations attempted (appendix F-M), the best calibration model for both whole and UDY kernel, and all traits was PLS_Constant_1D_SG_3-6. The number of factors used was selected by TQ Analyst

software through the use of the PRESS analysis. Each composition component had a different number of factors used based on the suggestions of the software.

Table 4. Summary statistics for calibration PLS_Constant_1D_SG_3-6 in whole kernel maize.

	RMSEC	r	PI	PLS factors
Crude protein %	0.37	0.98	70	10
Phosphorus %	0.02	0.94	60	8
Fat %	0.62	0.71	51	4
Starch %	1.89	0.86	29	7

Table 5. Summary statistics for calibration PLS_Constant_1D_SG_3-6 in UDY (1-mm) maize.

	RMSEC	r	PI	PLS factors
Crude protein %	0.35	0.98	90	9
Phosphorus %	0.03	0.88	63	10
Fat %	0.34	0.98	50	6
Starch %	1.95	0.85	13	8

Comparison between whole and ground kernel calibrations

The most accurate calibrations methods were those utilizing ground samples instead of whole samples. While utilizing the same prediction model (PLS_Constant_1D_SG_3-6), UDY maize samples had a better predictive ability for phosphorus (r=0.88, PI=63) compared to whole maize samples (r=0.94, PI=60) (Tables 4 and 5). The calibration was the most accurate for protein content in both whole (r=0.98,

PI=70) and ground (r=0.98, PI=90) kernel samples. Starch had the least predictive ability among all components in both its whole (r=0.86, PI=29) and UDY (r=0.85, PI=13) calibrations. Fat had similar PI values for both the whole kernel (r=0.71, PI=51) and UDY (r=0.98, PI=50) calibration with a slightly higher r value in the UDY calibration.

Better summary statistics for whole kernel versus UDY calibrations was an expected result of multiple factors including the absorbance/ loss/ reflectance properties of kernels vs. UDY powder as well as the homogenization of the samples through grinding. Light penetration limitations were also a likely contributing factor to differences between the calibrations. NIRS can only penetrate between 1-2 mm of a maize kernel with most kernels having an average thickness of 4-10 mm (Manley et al., 2009). As a result, kernel thickness generally exceeds light penetration in whole kernel samples, so one kernel may have the high oil embryo recorded and the neighboring kernel the high starch endosperm recorded. Therefore, the orientation of whole kernels in the NIR cup can greatly impact which area of the kernel is being analyzed; while the averaging in the rotating cup should help to alleviate this problem, it cannot eliminate it. The components looked at in this study vary in where they are stored within the kernel, in particular, the endosperm, embryo or aleurone layer. Therefore, through homogenization we reach a more accurate and consistent representation and reading of component concentrations in the sample. In grinding samples, it ensures the composition of the samples exposed to scanning are homogenized, it reduces the open areas for light to pass through, and it increases the numbers of kernels scanned.

Although the UDY calibration showed more accurate predictions according to the summary statistics, we believe that for a breeding program, utilization of the whole kernel calibration would be sufficient for most needs in choosing the extremes. Utilizing whole kernels keeps samples intact for future planting or other analyzing needs and no samples would have to go through the tedious and time consuming process of grinding.

Calibration and validation samples used in the whole kernel and UDY calibration model PLS_Constant_1D_SG_3-6 were evaluated through comparing NIR prediction values to the wet chemistry calculated values. Figure 3 compares whole kernel to UDY samples for crude protein. Values for the whole kernel model (R^2 = 0.92) are similar to that of the UDY calibration model (R^2 = 0.96). Whole kernel phosphorus had a slightly higher R^2 (0.82) value than did UDY phosphorus (R^2 =0.75) (Figure 4). There is a sharp increase in R^2 values when changing from whole kernel (R^2=0.49) to UDY samples (R^2 =0.85) (Figure 5). Whole kernel (R^2 =0.70) and UDY starch (R^2 =0.71) calibrations had nearly the same R^2 values.

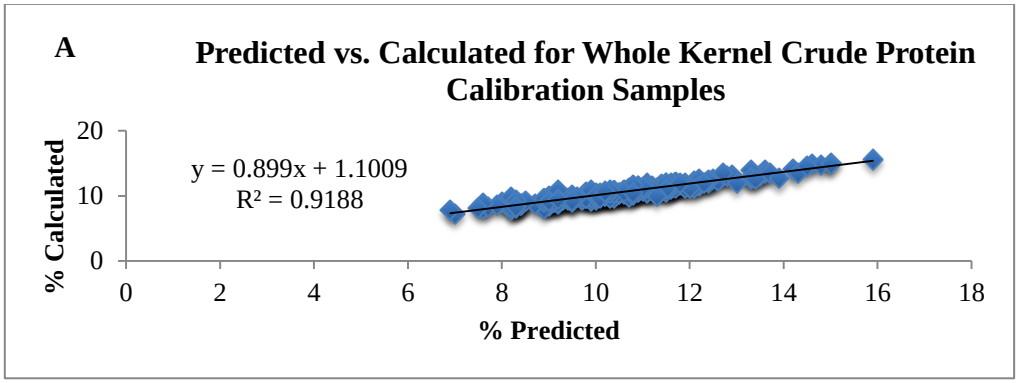


Figure 3. Linear model evaluating predicted and calculated values for calibration and validation samples for both (A) whole kernel and (B) UDY crude protein.

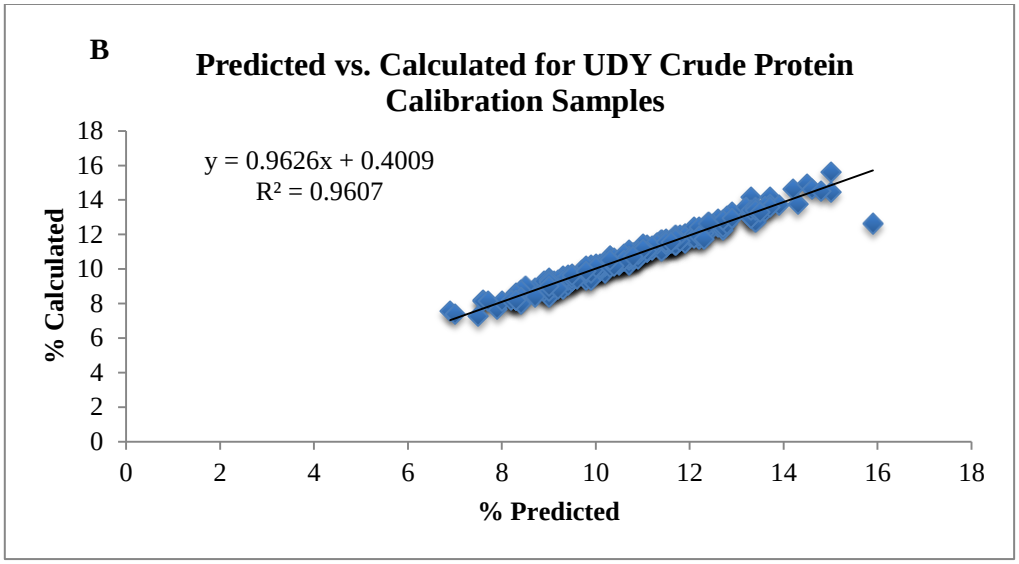


Figure 3 continued.

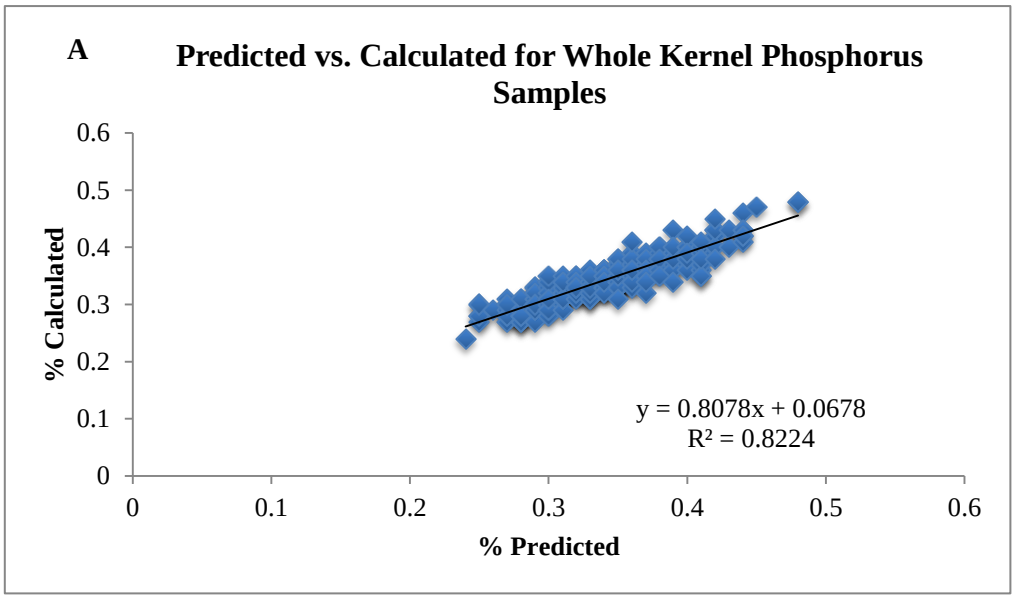


Figure 4. Linear model evaluating predicted and calculated values for calibration and validation samples for both (A) whole kernel and (B) UDY phosphorus.

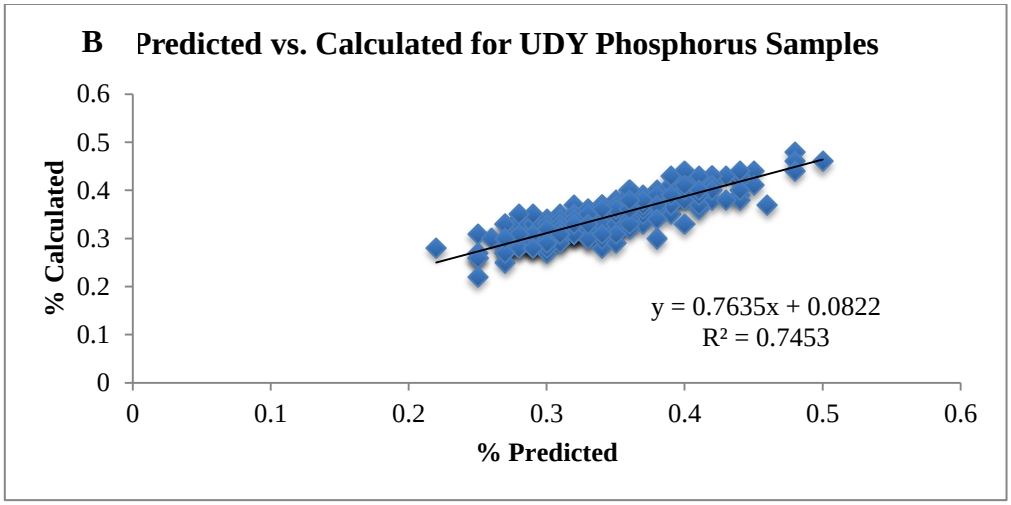


Figure 4 continued.

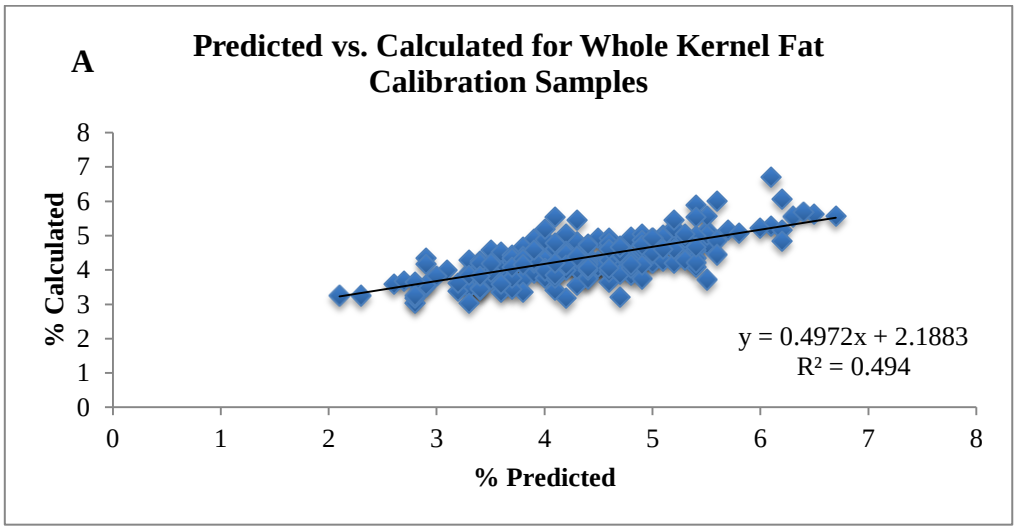


Figure 5. Linear model evaluating predicted and calculated values for calibration and validation samples for both (A) whole kernel and (B) UDY fat.

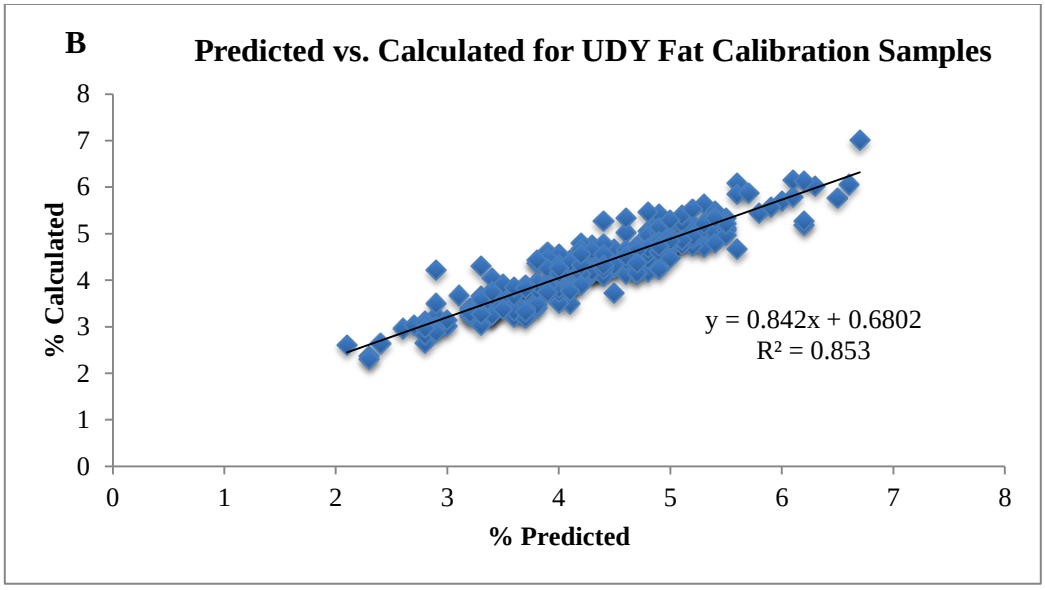


Figure 5 continued.

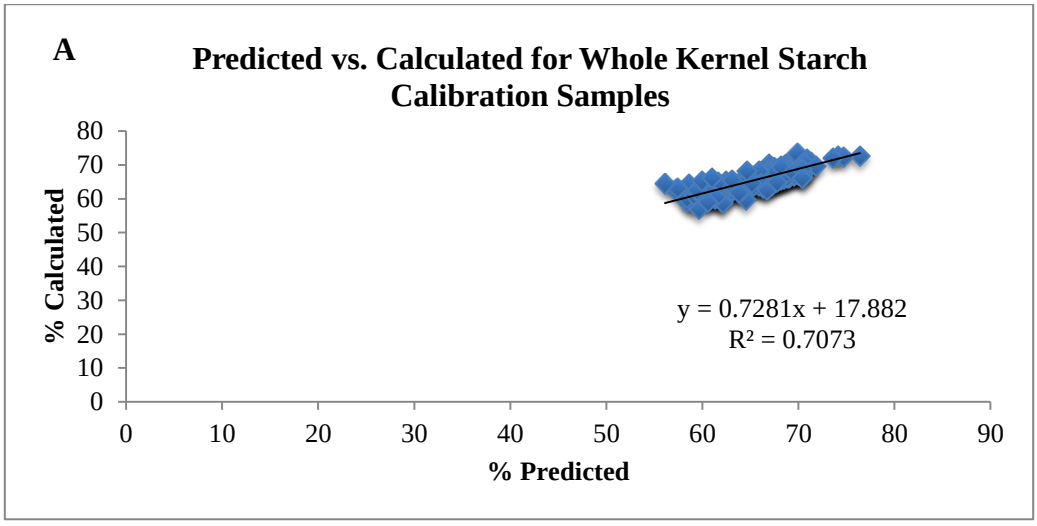


Figure 6. Linear model evaluating predicted and calculated values for calibration and validation samples for both (A) whole kernel (B) UDY starch.

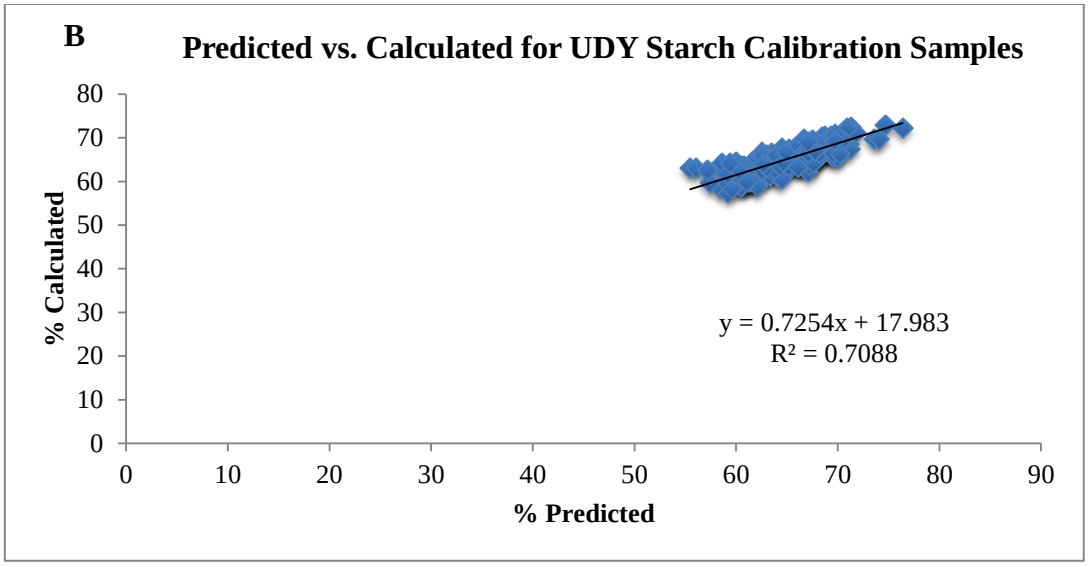


Figure 6 continued.

Accuracy vs. precision

Within the scope of this research project, our objective was to be able to utilize NIRS to accurately predict and rank samples for various composition components within a breeding program. Figure 7 below visually demonstrates the tradeoff between precision and accuracy. Calibration development can be approached in essentially one of two ways depending on which qualities in a trait are important to a breeding program. For some, samples being analyzed will come from similar or the same of environment and genotype. Consequently, the calibration will have a tendency towards high bias and low variance. For this experiment, samples grown in diverse environments and a myriad of genotypes were utilized. As a result, the calibration likely has low bias but high variance. We essentially sacrificed precision for all samples instead of accuracy for any

one experiment. With the continued addition of diverse samples as standards, the calibration will be able to better predict a wider array of sample types.

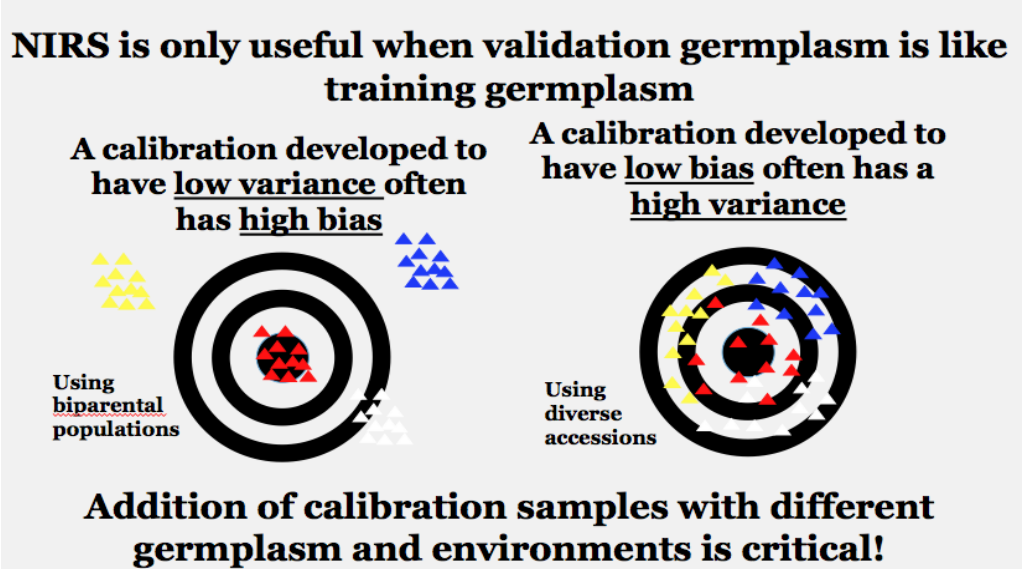


Figure 7. Precision vs. accuracy diagram.

CHAPTER V

IDENTIFYING AND VALIDATING DIVERSITY FOR HIGH AND LOW P

Introduction

One of the major advantages of NIRS, as opposed to wet-chemistry techniques, is the ability to develop calibrations and retrospectively predict the composition of samples that were scanned previously. The maize breeding program has routinely scanned thousands of samples from past studies for various ad-hoc research questions (Farfan et al. 2015, Meng et al. 2015, Mahan et al. 2013, Mahan et al. 2014 and Wahl et al. 2016 (submitted)). These historical TAMU NIRS spectra from samples collected between 2010-2015 were retrospectively predicted for P using the calibrations previously developed in Chapter IV for further analysis and breeding. The goal of this study was to identify natural genotypes that have high and low P for possible use in a breeding program and to genetically dissect grain P accumulation, preferably as hybrids.

Materials and Methods

Although the entire NIRS sample database was initially investigated, many genotypes were not replicated and a large environmental effect was observed. Therefore a subset of samples from Farfan et al. (2015) was focused on. A total of 2,185 whole kernel samples with 345 diverse genotypes (scanned previously) were predicted for P content based on the calibration developed in Chapter V. The samples were matched with pedigree information and put into a model to get BLUPS using JMP software. The output was analyzed to find extreme high and low phosphorus levels and pedigrees that consistently fell into the same category. The samples selected for planting were

rescanned with a newer calibration model to verify extreme P levels. P levels of samples selected for planting ranged between 0.27%-0.43%. Any number below 0.35% was considered low P and anything above 0.35% was considered high P. Sample predictions included inbreds and hybrids. In the case of a hybrid being identified with a low or high level of P, both parents were included in the crossing block where seed was available.

Sources of diverse genotypes were obtained primarily as nursery stocks kept by the Texas A&M Quantitative Genetics and Maize Breeding Program. These were planted in a crossing block at the TAMU Research Center in Weslaco, Texas. Two successive plantings occurred on August 6th and 13th, 2015. Two 20 ft. plots per inbred were planted on 40 inch centers at a rate of 25 seeds per plot (16,335 seeds/acre). Plants were crossed in a high P x high P, high P x low P, low P x high P and low P x low P design. In addition, within each row, several plants were selfed, while the remainder were allowed to open pollinate.

The seed was hand harvested on December 16, 2015 and dried to approximately 15% moisture. The whole kernel samples were analyzed using NIRS. The NIRS calibrations predicted phosphorus, crude protein, starch and fat levels in the samples. The grain was analyzed to determine if the pollen donor had effects on P concentration in the grain, known as the 'xenia' effect. This is important for future studies where many varieties will be grown adjacent to each other. Additionally, we wanted to evaluate the ability of the developed calibrations, particularly whole kernel, to rank P levels of harvested samples.

Spectral predictions were analyzed to determine whether P levels differed between selfed, open-pollinated samples or crossed samples. This was followed by wet-chemistry analysis as explained in Chapter 4 to verify the results of the NIR predictions.

Statistical Analysis

A Students T-Test was performed in JMP (APPENDIX N.) at the onset of the research to identify genotypes with consistently high and low kernel P concentrations from the database, mostly from the study of Farfan et al. 2015 (Table 6). Additional samples were selected from the study of Anderson 2016 since many of the stocks from Farfan were no longer available.

Genotypes and tests nested within environments corresponding to each sample were added so that the data could be analyzed for the basis of variation and that samples with extreme high and low P levels could be identified. A simple model was fit: Predicted P = genotype + test [environment] + error.

A list of the preliminarily selected lines is found in APPENDIX N. Samples were selected using a truncation selection from the top and bottom set of P levels. It would have been confounding to include both inbreds and hybrids in further analysis and in many cases the hybrid seed stocks were not available so only the inbred lines from the hybrids were selected. Not all extreme genotypes were selected because they were known to be poorly adapted, there was only one observation or conflicting observations, and or in some cases the inbred seed stocks were no longer available.

Table 6 shows the selected inbred genotypes and the stocks available for planting along with the composition component estimates from NIRS. Two different NIRS

calibrations, the best P calibration and another calibration that also predicts P, were used to diversify investment in any one set of samples. This was done because the samples of interest were intentionally rare and at the extremes of the distribution and it is not possible to know how well any one model works on the extremes. According to NIRS calibration Whole-4-Cp (Table 2), which was selected for best predicting crude protein in whole kernel grain, GT112 had the lowest P level at 0.27% and Tx772/Tx906-4-3-1-1-1-B8 had the highest P level at 0.43%. Within NIRS calibration Whole-4-PFS, which best predicts phosphorus, fat, and starch, CMV3 and CS13-APOPN-237 both had the lowest level of P with 0.31%. CS13-BPOPN-439 and NC348 both had the highest level of P with 0.45%.

Table 6. NIRS predictions for inbred genotypes selected for field study. Stocks planted in Weslaco for crossing and predictions of their composition from two FT-NIRS calibrations. Predictions shown are from rescanning of the original sample.

		Calibration Whole-4-Cp NIRS predictions for stock samples				**Calibration Whole-4-PFS NIRS predictions for stock samples**			
Genotype	Stock	Crude protein	Phosphorus	Fat	Starch	Crude protein	Phosphorus	Fat	Starch
(B73 Oleic/Tx903)/(Tx772/Tx906)-Cs10#Group4B-6#-4#-2-1-1-1-B6	CS14-STEVE-188-B6	10.98	0.33	4.35	66.64	10.97	0.32	4.03	67.40
(P69Qc3HC107-1-1#-4-2#-4-B-B-1-4-B-B-B-B-B/CML193)-B-B-2-B-B-B-B-1-B26-B9	CS13-INC-140-B9								
(Tx802/Ko326y)-18-1-1-1-B-B/CML161-B-4-B-B-B-B-1-B22-B7	CS13-INC-136-B7	12.64	0.35	4.50	63.20	12.30	0.34	4.28	64.15
(Tx811); ((Ko326y x Tx806)-6-1-1-1-B-B/CML161)x(Tx802/CML161))-2-B-B-B-B-1-B15-B13	CS14-INC-073-B13	12.68	0.39	4.83	60.90	11.84	0.37	4.03	62.52
CMV3	CS14-MENG-001	13.13	0.31	4.49	64.72	12.35	0.31	4.16	66.30
CS13-APOPN-237	CS14-STEVE-410-1	10.64	0.32	4.71	66.13	10.27	0.31	4.40	66.56
CS13-BPOPN-439	CS14-STEVE-003-B5	13.06	0.39	5.09	56.96	12.66	0.45	4.65	59.51
GT112	CS14-MENG-203	12.03	0.27	4.95	65.56	11.36	0.33	4.78	66.43
LaPoSTaSEqC7-F102-1-3-1-1-B-B-B-B13	CS14-MENG-278	13.68	0.32	4.89	62.67	12.76	0.33	4.52	63.46
Mp04:97	CS14-MENG-256	13.27	0.40	4.58	60.77	12.88	0.43	4.59	61.64

Table 6 continued.

		Calibration Whole-4-Cp NIRS predictions for stock samples				**Calibration Whole-4-PFS NIRS predictions for stock samples**			
Genotype	Stock	Crude protein	Phosphorus	Fat	Starch	Crude protein	Phosphorus	Fat	Starch
NC222	CS14-MENG-220-B6	13.05	0.38	5.04	59.43	13.11	0.43	4.32	61.49
NC348	CS14-MENG-213-B8	13.49	0.38	5.80	59.60	13.47	0.45	5.24	60.91
NC370	CS14-MENG-241	11.56	0.30	3.96	68.04	11.50	0.33	4.72	67.39
S2B73	CS14-MENG-316	12.41	0.40	4.49	62.87	12.41	0.41	4.25	64.09
Tx772/Tx906-4-3-1-1-1-B8	CS14-STEVE-382-B8	11.54	0.43	5.25	63.34	12.24	0.40	4.57	64.18
Minimum		10.64	0.27	3.96	56.96	10.27	0.31	4.03	59.51
Maximum		13.68	0.43	5.80	68.04	13.47	0.45	5.24	67.40
Mean		12.44	0.36	4.78	62.92	12.15	0.37	4.47	64.00

Results and Discussion

The results of the wet chemistry analysis were largely consistent with expectations based on the NIRS predictions. On a whole, samples harvested in the Weslaco 2016 winter nursery (referred to as PJCB samples) tended to have higher average P content than those evaluated from other environments in the database. This is consistent with the preliminary analysis described earlier in the chapter.

From a visual observation of comparing PJCB wet chemistry results to PJCB NIRS predictions from both whole kernel and UDY calibrations (Table 7) it was apparent that the UDY calibration on a whole was a better predictor of actual values than the whole kernel calibration. Samples originally predicted to be low P in most cases were instead shown to be high P Samples originally predicted to be high P based on the parental stock stayed true to the high P characteristic.

Specifically, GT112 was predicted to be low and was low in PJCB samples, Mp04:97 was predicted to be high and was high in PJCB samples. However, Tx811 was predicted to be low, but was high and NC348 was predicted to be low but was high. This suggests either an environmental effect on the genotype or that the calibrations are not really as predictive as believed, especially for identifying promising outlier genotypes.

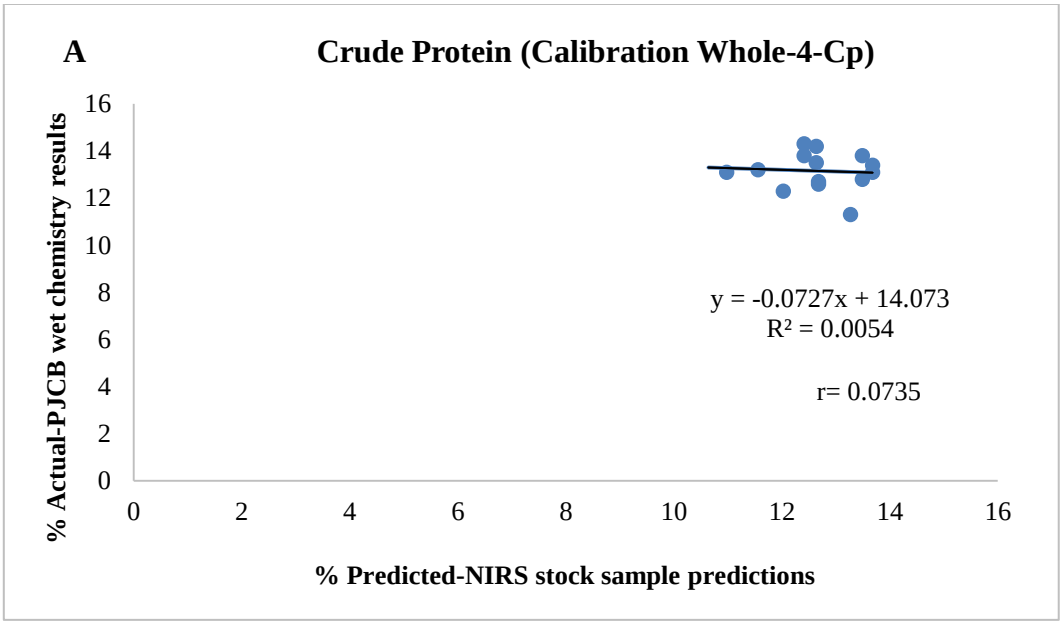
Table 7. NIRS predictions and wet chemistry results for field experiment samples.

				PJCB whole kernel NIRS values				PJCB UDY grind NIRS values				PJCB wet chemistry results			
Sample ID	**Pedigree**	**P Level**	**Treatment**	**CP**	**Phos**	**Fat**	**Starch**	**CP**	**Phos**	**Fat**	**Starch**	**CP**	**Phos**	**Fat**	**Starch**
PJCB-2-OP	GT112-B14	Low	OP	11.23	0.37	5.47	63.54	12.44	0.34	4.39	71.80	12.30	0.30	4.30	68.40
PJCB-2-X-PJCB-12	GT112-B14/NC222-B6	Low X High	Cross	12.17	0.38	4.99	63.59	11.31	0.38	4.41	70.45	12.10	0.32	4.00	66.90
PJCB-3-OP	NC370-B27-OP16	Low	OP	11.49	0.36	4.69	64.95	12.24	0.47	6.30	64.04	13.20	0.39	4.80	68.80
PJCB-3-X-PJCB-12	NC370-B27/NC222-B6	Low X High	Cross	12.39	0.39	5.32	64.40	12.84	0.38	4.93	67.63	13.50	0.39	4.70	64.10
PJCB-3-X-PJCB-13	NC370-B27/Mp04:97-B20	Low X High	Cross	11.91	0.36	4.85	63.60	14.30	0.44	5.21	67.92	14.70	0.40	4.60	63.20
PJCB-4-OP	LaPoSTaSEqC7-F102-1-3-1-1-B-B-B-B13	Low	OP	11.78	0.37	4.78	65.59	11.94	0.41	4.22	69.31	13.40	0.41	4.10	66.00
PJCB-4-B4	LaPoSTaSEqC7-F102-1-3-1-1-B-B-B-B13	Low	Self	11.88	0.37	4.47	63.93	12.12	0.39	4.17	67.97	13.10	0.39	3.30	62.20
PJCB-5-OP	(B73 Oleic/Tx903)/(Tx772/Tx906)-Cs10#Group4B-6#-4#-2-1-1-1-B6-OP3	Low	OP	10.29	0.35	4.96	67.47	11.42	0.40	4.79	67.96	13.10	0.35	3.10	63.80
PJCB-7-OP	(Tx802/Ko326y)-18-1-1-1-B-B/CML161-B-4-B-B-B-B-1-B22-B7-OP2	Low	OP	13.24	0.40	4.87	62.97	13.20	0.43	5.50	65.38	14.20	0.41	5.30	62.00
PJCB-7-B4	(Tx802/Ko326y)-18-1-1-1-B-B/CML161-B-4-B-B-B-B-1-B22-B7-B4	Low	Self	12.51	0.37	4.56	61.80	12.04	0.43	5.30	63.66	13.50	0.43	5.40	61.20
PJCB-8-OP	(Tx811); ((Ko326y x Tx806)-6-1-1-1-B-B/CML161)x(Tx802/CML161))-2-B-B-B-B-1-B15-B13-OP3	Low	OP	11.68	0.36	4.25	64.01	11.54	0.38	4.09	66.53	12.70	0.45	3.70	66.40

Table 7 continued.

				PJCB whole kernel NIRS values				PJCB UDY grind NIRS values				PJCB wet chemistry results			
Sample ID	**Pedigree**	**P level**	**Trt.**	**CP**	**Phos**	**Fat**	**Starch**	**CP**	**Phos**	**Fat**	**Starch**	**CP**	**Phos**	**Fat**	**Starch**
PJCB-8-X-PJCB-17	(Tx811); ((Ko326y x Tx806)-6-1-1-1-B-B/CML161)x(Tx802/CML161))-2-B-B-B-B-1-B15-B13/(P69Qc3HC107-1-1#-4-2#-4-B-B-1-4-B-B-B-B-B/CML193)-B-B-2-B-B-B-B-1-B26-B9	Low X High	Cross	11.30	0.37	4.47	63.45	11.26	0.41	5.01	65.70	12.20	0.44	4.30	64.30
PJCB-11-B4	S2B73-.-B4	High	Self	12.46	0.38	4.51	61.83	13.24	0.48	5.18	65.98	13.80	0.40	4.40	62.60
PJCB-11-OP	S2B73-.-OP	High	OP	11.39	0.37	4.79	63.18	13.61	0.48	5.33	66.51	14.30	0.46	5.10	60.40
PJCB-13-B2	Mp04:97-B20-B2	High	Self	12.10	0.38	5.00	65.15	10.85	0.39	4.13	70.13	11.30	0.45	3.70	67.20
PJCB-13-X-PJCB-3	Mp04:97-B20/NC370-B27	High X Low	Cross	11.60	0.39	5.62	64.61	11.12	0.41	4.95	66.18	11.60	0.44	4.50	65.00
PJCB-14-OP	NC348-B8-OP2	High	OP	12.33	0.39	4.93	61.85	12.13	0.41	3.51	66.57	12.80	0.30	2.30	62.70
PJCB-14-B2	NC348-B8-B5	High	Self	12.94	0.40	4.91	61.00	13.08	0.44	3.24	64.05	13.80	0.35	2.40	60.30
PJCB-17-B3	(P69Qc3HC107-1-1#-4-2#-4-B-B-1-4-B-B-B-B-B/CML193)-B-B-2-B-B-B-B-1-B26-B9-B4	High	Self	12.32	0.40	5.13	61.38	10.92	0.43	4.78	65.61	12.30	0.38	4.60	65.10
PJCB-16-B3	Tx772/Tx906-4-3-1-1-1-B8-B3	High	Self	12.80	0.41	5.11	61.89	*	*	*	*				
Minimum				10.29	0.35	3.86	59.48	10.85	0.34	3.24	63.66	11.30	0.30	2.30	60.30
Maximum				13.24	0.41	5.62	67.47	14.30	0.48	6.30	71.80	14.70	0.46	5.40	68.80
Mean				11.96	0.38	4.83	63.34	12.13	0.41	4.68	66.91	13.03	0.40	4.13	64.55

Correlations were made between the stock sample NIR predictions and the wet chemistry results from PJCB samples. When comparing stock sample NIR predictions for crude protein to the PJCB wet chemistry results, a very weak correlation was observed for both calibration models. Calibration Whole-4-Cp had an r value of 0.07 while Whole-4-PFS had a higher value of 0.11 (Figure 8). The r value for phosphorus using calibration Whole-4-Cp is 0.50 whereas the r value was 0.02 for calibration Whole-4-PFS (Figure 9). Fat showed the strongest correlation of all four components with an r of 0.74 for calibration Whole-4-Cp and an r of 0.50 for calibration Whole-4-PFS (Figure 10). Starch had similar correlation coefficients with 0.45 for Whole-4-Cp and an r value of 0.40 for Whole-4-PFS (Figure 11).



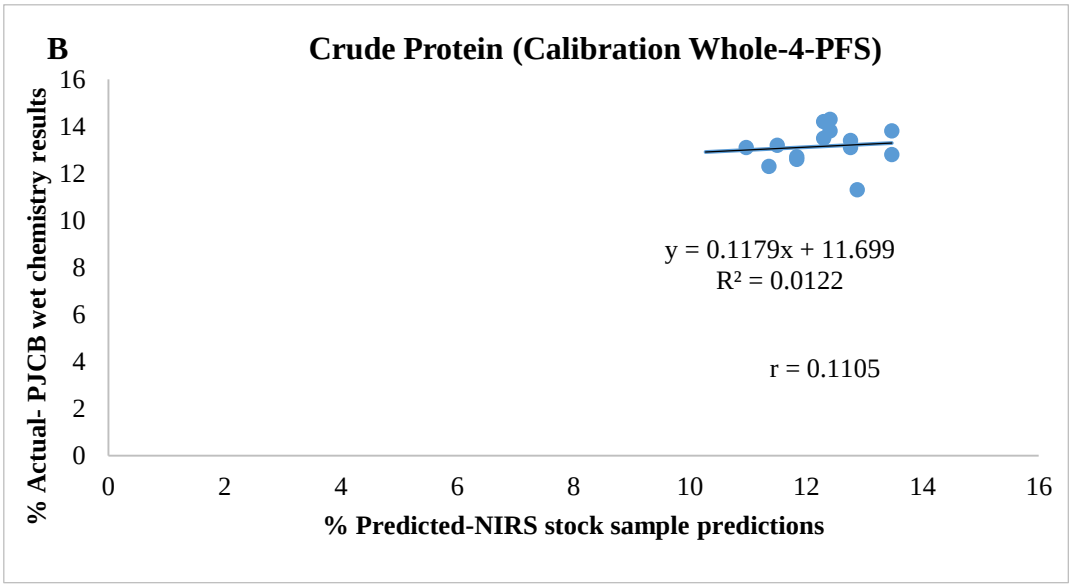
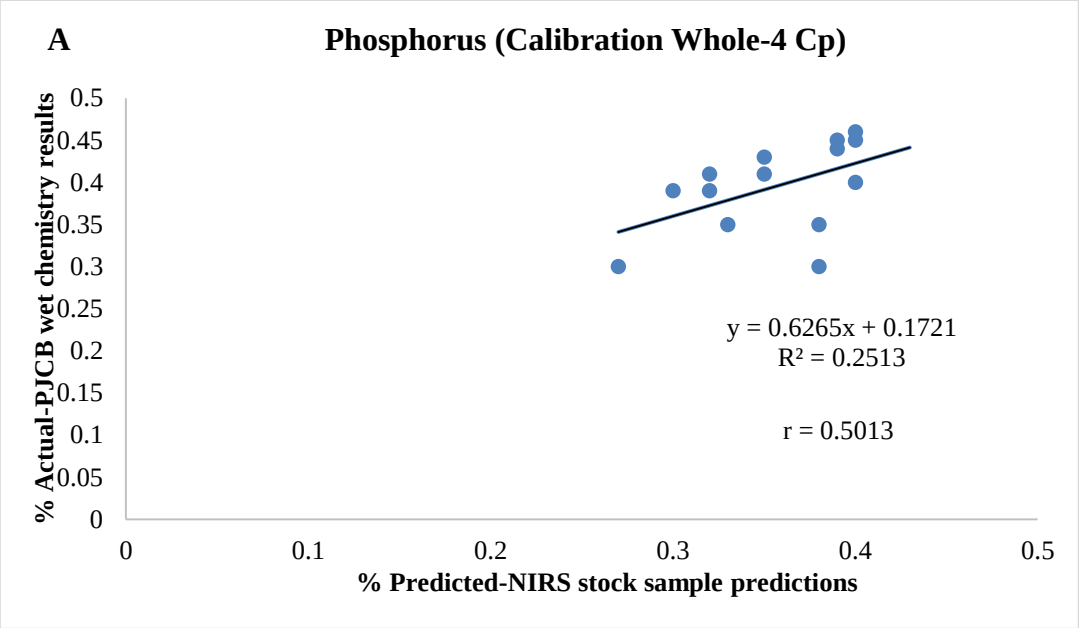


Figure 8. Correlation of percent crude protein between whole kernel stock sample predictions and PJCB wet chemistry results utilizing calibration Whole-4-Cp (A) and Whole-4-PFS (B).



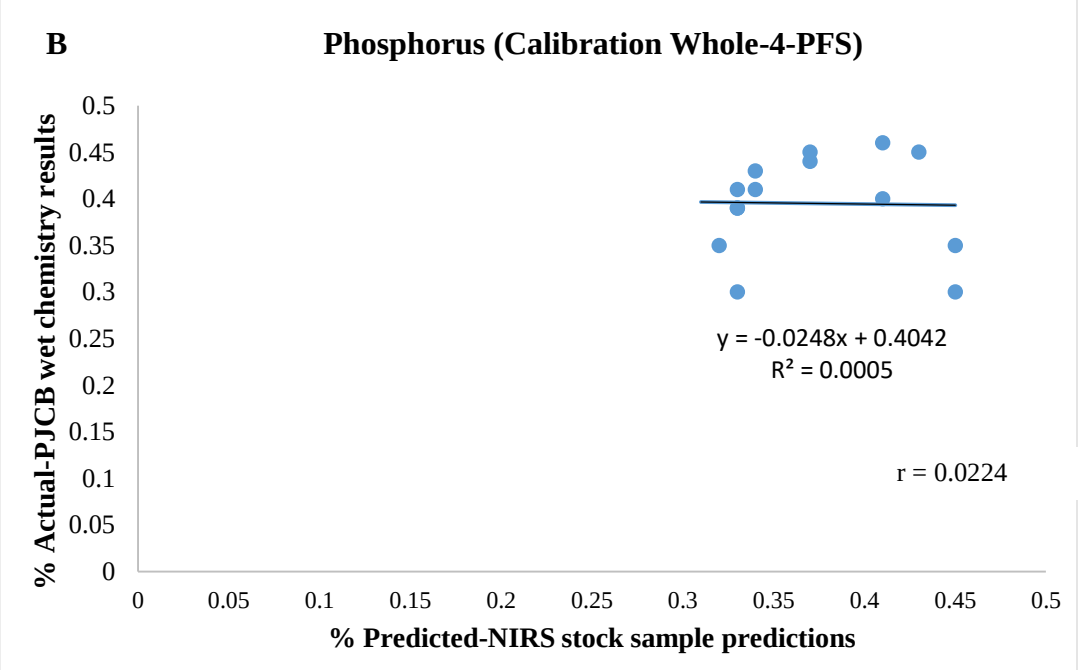
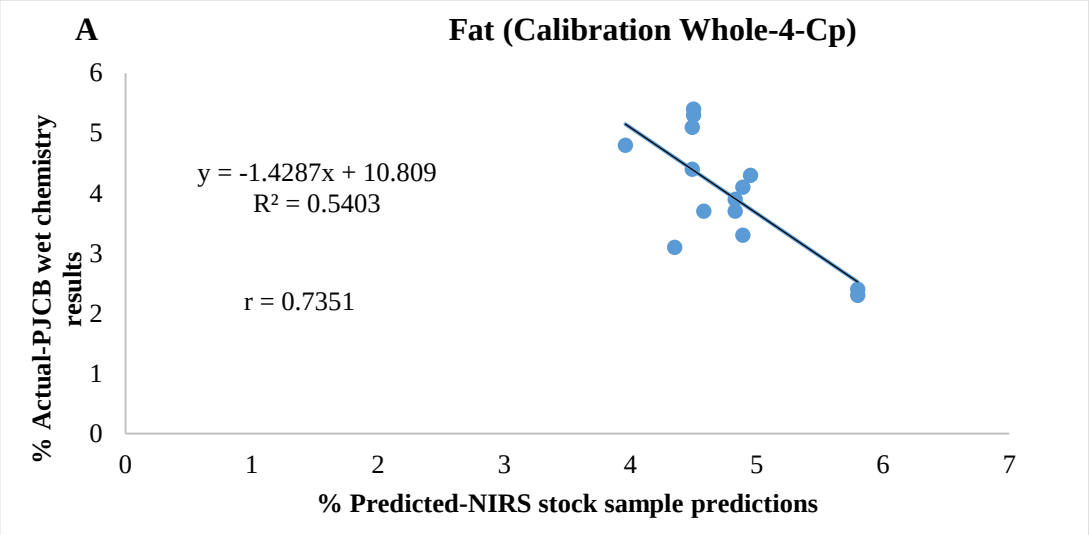


Figure 9. Correlation of percent phosphorus between whole kernel stock sample predictions and PJCB wet chemistry results utilizing calibration Whole-4-Cp (A) and Whole-4-PFS (B).



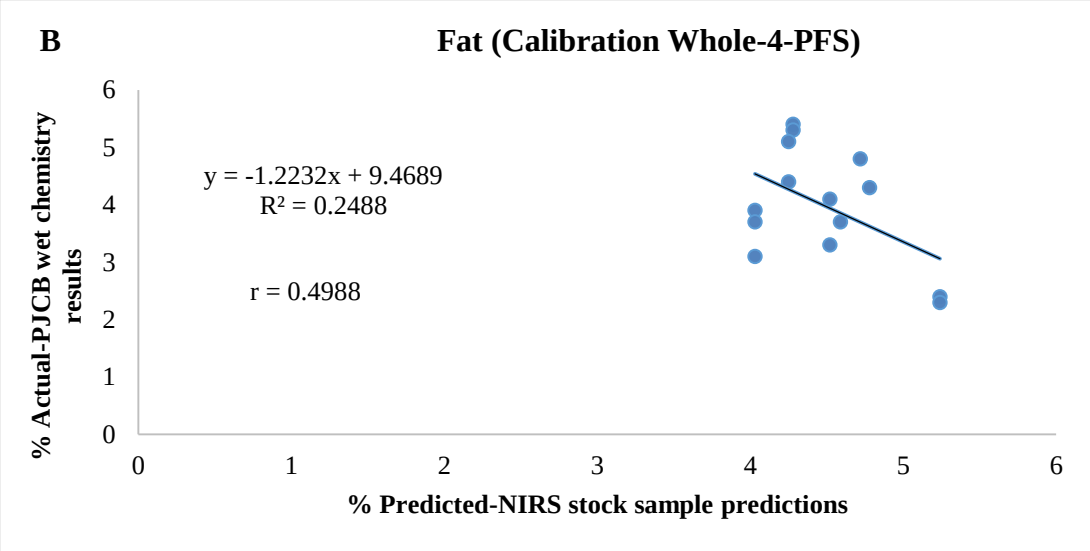


Figure 10. Correlation of percent fat between whole kernel stock sample predictions and PJCB wet chemistry results utilizing calibration Whole-4-Cp (A) and Whole-4-PFS (B).

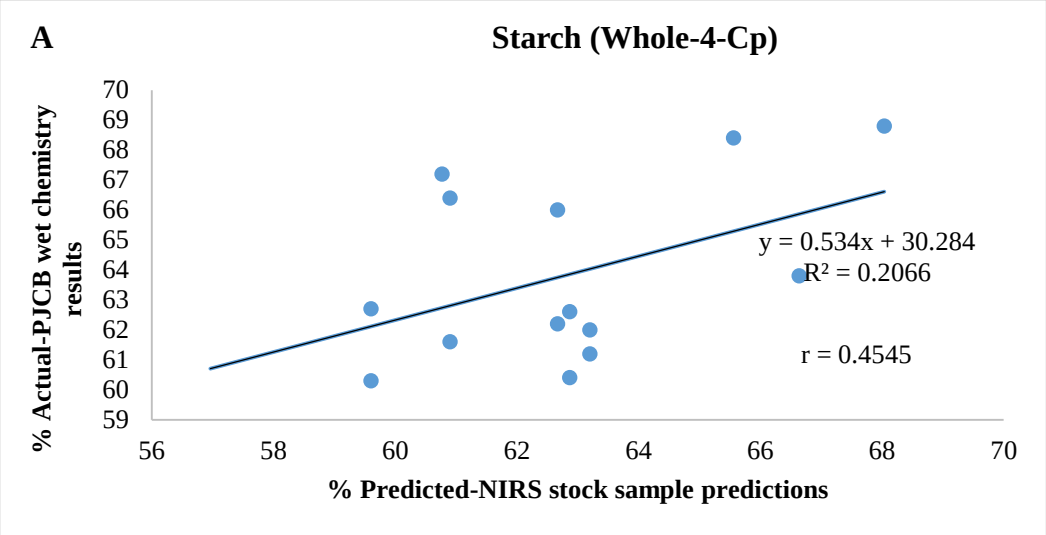
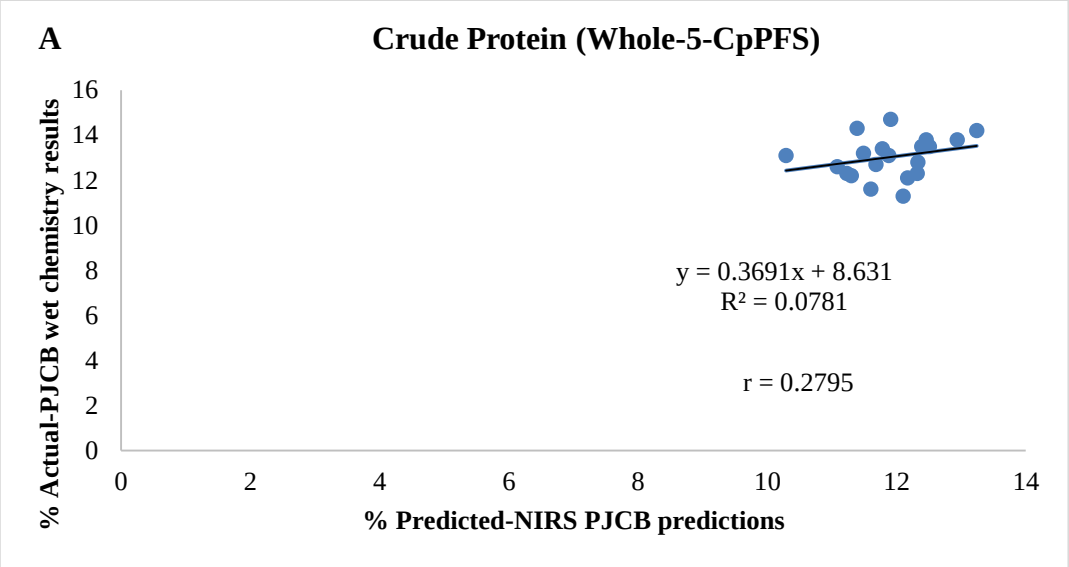


Figure 11. Correlation of percent starch between whole kernel stock sample predictions and PJCB wet chemistry results utilizing calibration Whole-4-Cp (A) and Whole-4-PFS (B).

The PJCB NIR predictions were compared to their subsequent wet chemistry results to evaluate the effectiveness of the calibrations to predict the actual component values (Figures 12 and 13). While whole kernel crude protein had smaller r value of 0.28, UDY had a high r value of 0.88. R values for both whole kernel (r = 0.19) and

UDY (r = 0.28) phosphorus, although positive, were similar to whole kernel crude protein. However for phosphorus this could have been due to the narrower than expected range for these samples. Although whole kernel fat had a small r value of 0.07, UDY fat had a much higher correlation value of 0.83. Whole kernel and UDY starch had similar r values of 0.46 and 0.53, respectively.

The correlations between PJCB whole kernel predictions and wet chemistry results were not as high in some cases as would be ideal. Trends were consistent with what we would expect when comparing whole kernel predictions with those from UDY samples. Again, homogenization of the sample and kernel orientation, play a large part in the differences between the calibrations. Several additional factors could be attributed to smaller correlation values, but mainly a small sample size within a poor nursery. A small number of plots were harvestable due to poor growth and low seed set thus reducing the number of samples we could evaluate. There was not enough statistical evidence from this particular field trial to prove that the NIR calibrations could accurately predict the components of interest. The addition of more growing locations and replicates, along with higher quality samples would hopefully lend enough statistical evidence to be confident in whether the NIR calibrations could accurately validate P levels in both whole and UDY maize.



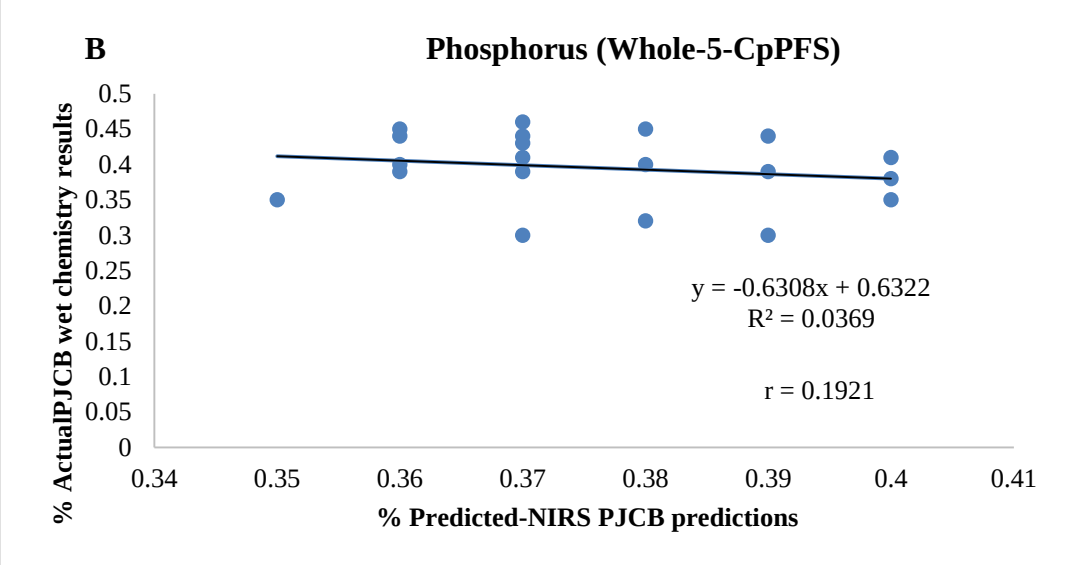
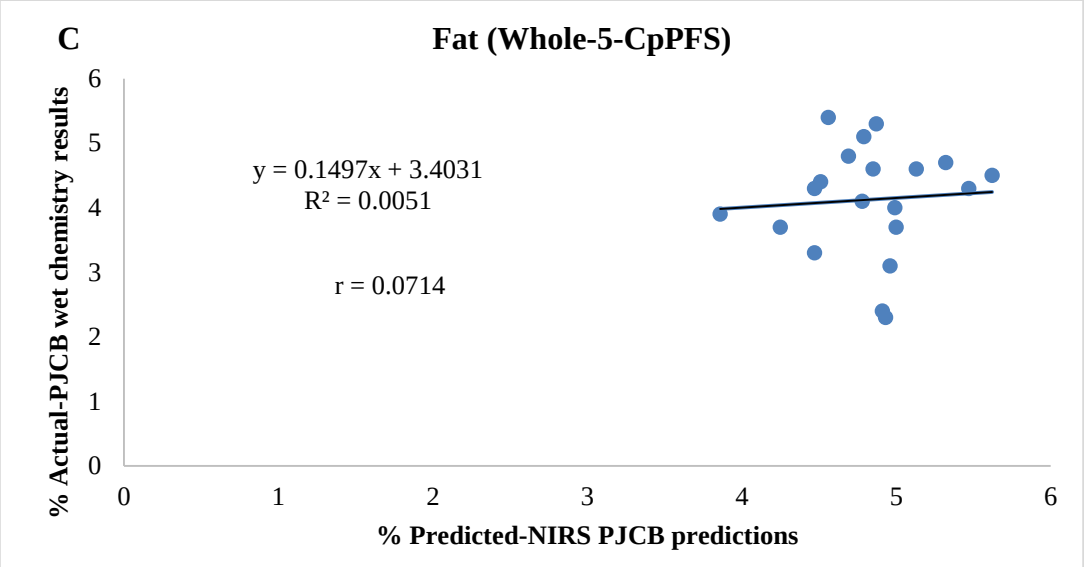
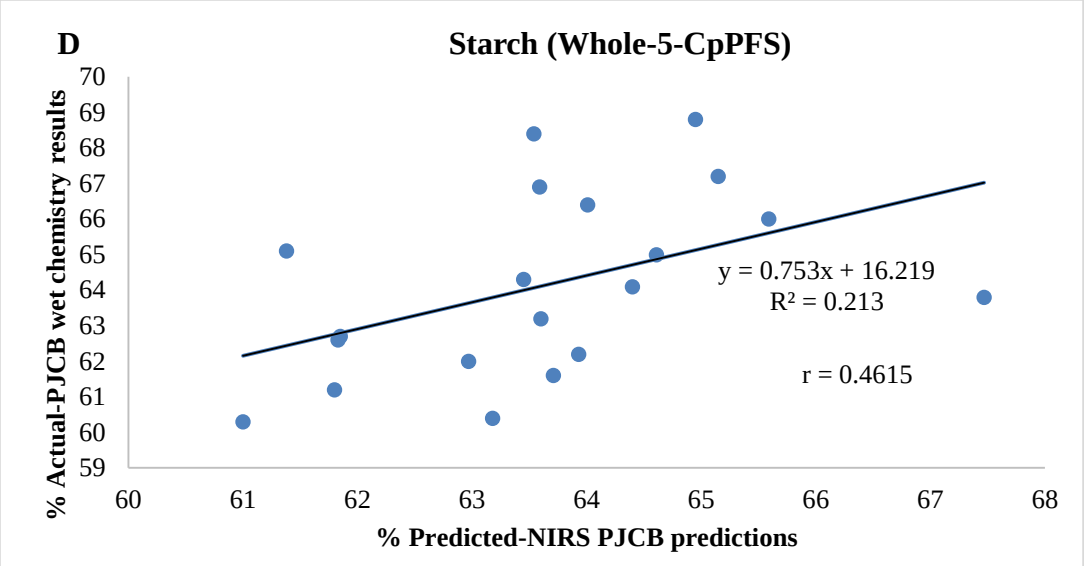


Figure 12. Correlation between whole kernel PJCB samples and Weslaco wet chemistry results for (A) crude protein, (B) phosphorus, (C) fat, and (D) starch





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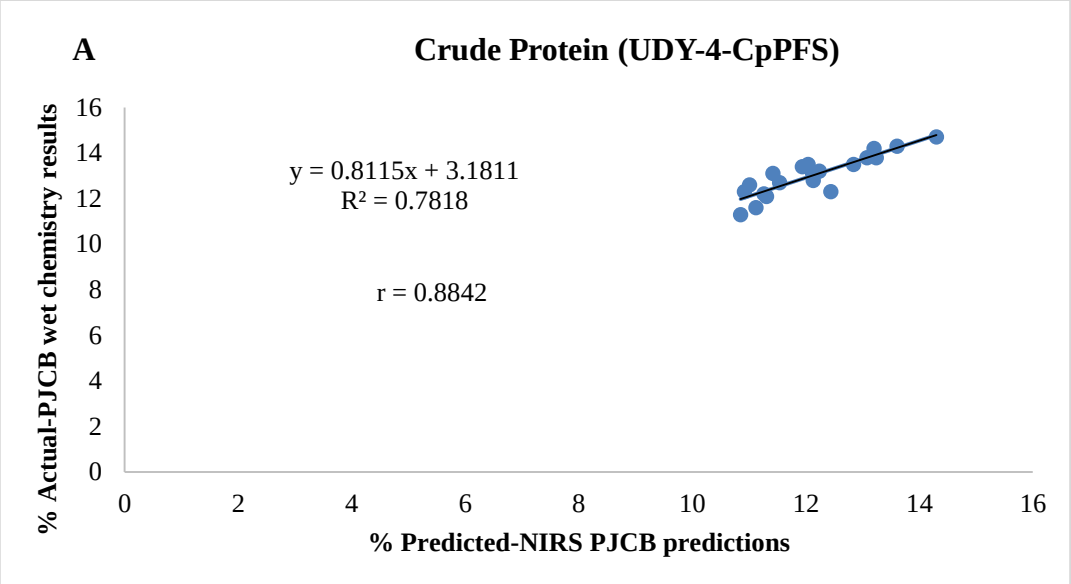


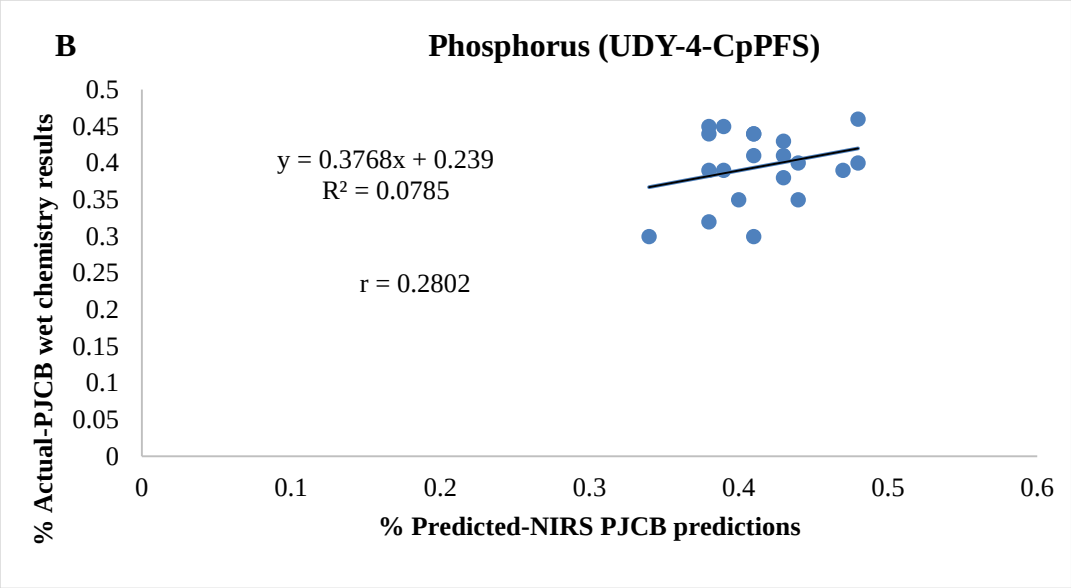
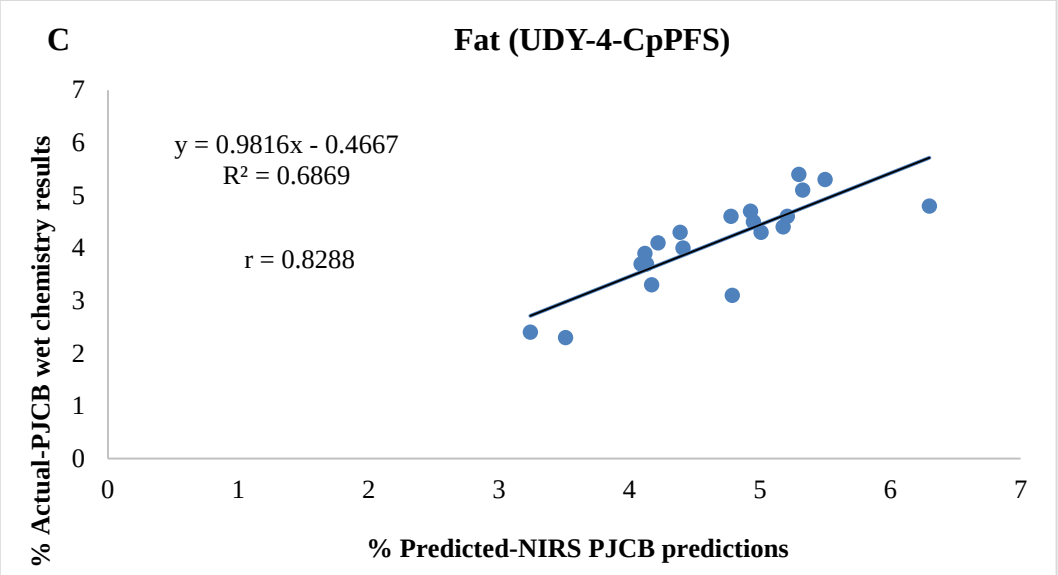


Figure 13. Correlation between UDY PJCB samples and PJCB wet chemistry results for (A) crude protein, (B) phosphorus, (C) fat, and (D) starch.



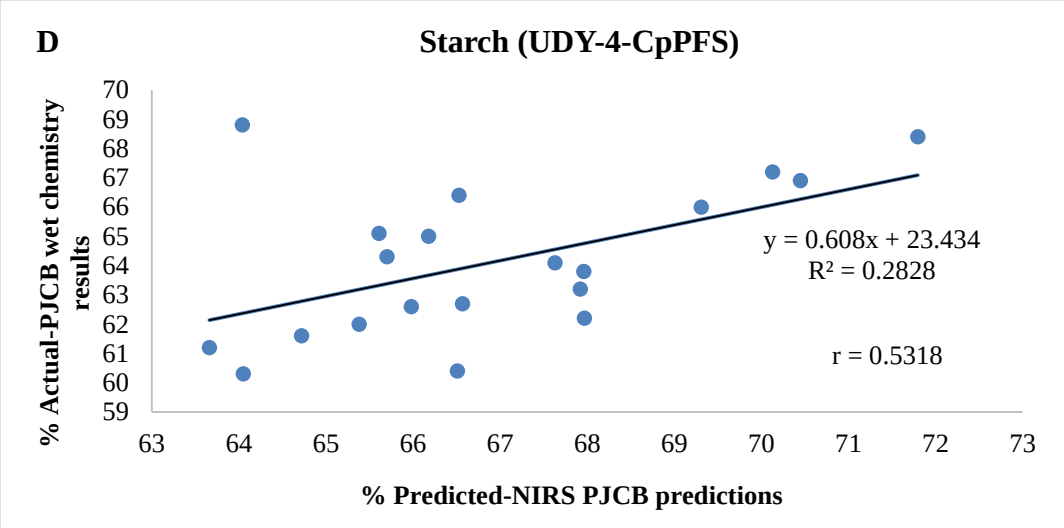


Figure 13 continued.

Ranking P levels

A major objective of this study was to develop and validate a calibration that could properly rank P concentrations in maize samples. Table 8 lists P levels according to our original criteria for P level classification with low P samples being those below 0.35% and high samples anything above 0.35%. The table shows predicted P levels for the parental stock from which Weslaco samples were obtained. Both whole kernel and UDY calibrations mostly correctly ranked P levels in accordance with the wet chemistry analysis results. The only exception to this were with the genotypes GT112 and NC348. Open pollinated and crossed GT112 samples were predicted to have high P levels by both whole and UDY calibrations when they were actually low P according to chemistry results; it is interesting and important to notice that GT112 was selected as a parent because it was expected to be low. NC 348 open pollinated and selfed samples followed the same pattern as GT112, being predicted as high P when they were in fact low P; however in this case NC348 was selected as a parent because it was correctly expected to be high. (B73 Oleic/Tx903)/ (Tx772/Tx906) was correctly classified by the whole kernel calibration but not the UDY calibration.

Table 8. Classification of P levels in Weslaco samples.

Sample ID	Genotype	Treatment	Parental P level	P level whole kernel prediction	P level UDY prediction	Weslaco P level (wet chemistry)
WF15-PJCB-2-OP	GT112	OP	Low	High	Low	Low
WF15-PJCB-2-X-PJCB-12	GT112/NC222	Cross	Low X High	High	High	Low
WF15-PJCB-3-OP	NC370	OP	Low	High	High	High
WF15-PJCB-3-X-PJCB-12	NC370/NC222	Cross	Low X High	High	High	High
WF15-PJCB-3-X-PJCB-13	NC370/Mp04:97	Cross	Low X High	High	High	High
WF15-PJCB-4-OP	LaPoSTaSEqC7-F102	OP	Low	High	High	High
WF15-PJCB-4-B4	LaPoSTaSEqC7-F102	Self	Low	High	High	High
WF15-PJCB-5-OP	(B73 Oleic/Tx903)/(Tx772/Tx906)-Cs10#Group4B-6#-4#-2-1-1-1-B6-OP3	OP	Low	Low	High	Low
WF15-PJCB-7-OP	(Tx802/Ko326y)-18-1-1-1-B-B/CML161-B-4-B-B-B-B-1-B22-B7-OP2	OP	Low	High	High	High
WF15-PJCB-7-B4	(Tx802/Ko326y)-18-1-1-1-B-B/CML161-B-4-B-B-B-B-1-B22-B7-B4	Self	Low	High	High	High
WF15-PJCB-8-OP	(Tx811); ((Ko326y x Tx806)-6-1-1-1-B-B/CML161)x(Tx802/CML161))-2-B-B-B-B-1-B15-B13-OP3	OP	Low	High	High	High
WF15-PJCB-8-B7	(Tx811); ((Ko326y x Tx806)-6-1-1-1-B-B/CML161)x(Tx802/CML161))-2-B-B-B-B-1-B15-B13-B7	Self	Low	High	High	High
WF15-PJCB-8-X-PJCB-17	(Tx811); ((Ko326y x Tx806)-6-1-1-1-B-B/CML161)x(Tx802/CML161))-2-B-B-B-B-1-B15-B13/(P69Qc3HC107-1-1#-4-2#-4-B-B-1-4-B-B-B-B-B/CML193)-B-B-2-B-B-B-B-1-B26-B9	Cross	Low X High	High	High	High

Table 8 continued

Sample ID	Genotype	Treatment	Parental P level	P level whole kernel prediction	P level UDY prediction	Weslaco P level (wet chemistry)
WF15-PJCB-11-B4	S2B73	Self	High	High	High	High
WF15-PJCB-11-OP	S2B73	OP	High	High	High	High
WF15-PJCB-13-B2	Mp04:97	Self	High	High	High	High
WF15-PJCB-13-X-PJCB-3	Mp04:97/NC370	Cross	High X Low	High	High	High
WF15-PJCB-14-OP	NC348	OP	High	High	High	Low
WF15-PJCB-14-B2	NC348	Self	High	High	High	Low
WF15-PJCB-16-B3	Tx772/Tx906-4-3-1-1-1-B8-B3	Self	High	High	*	*
WF15-PJCB-17-B3	(P69Qc3HC107-1-1#-4-2#-4-B-B-1-4-B-B-B-B-B/CML193)-B-B-2-B-B-B-B-1-B26-B9-B4	Self	High	High	High	High
WF15-PJCB-17-X-PJCB-8	(P69Qc3HC107-1-1#-4-2#-4-B-B-1-4-B-B-B-B-B/CML193)-B-B-2-B-B-B-B-1-B26-B9/(Tx811); ((Ko326y x Tx806)-6-1-1-1-B-B/CML161)x(Tx802/CML161))-2-B-B-B-B-1-B15-B13	Cross	High X Low	High	*	*

Interestingly, the majority of genotypes grown in Weslaco regardless of breeding treatment and parental stock P level, were of a higher P level than expected. Several possible factors (outside the scope of this project) could contribute to high P levels, including soil type, environmental effects, and seed quality. The 2015 growing season presented growing challenges due to record precipitation events. As a result, flooding was a frequent occurrence at the Weslaco research center. From August 2015-October 2015, the station recorded 20.33 inches of rain with 10.02 inches alone falling in October, a crucial time in seed development (southtexasweather.tamu.edu). The PJCB research plots saw spotty germination and stunted growth. Consequently, seed set and quality were negatively affected. Due to time and resource constraints, we were only able to gather data from this one Weslaco winter nursery. However, it would be our suggestion for further studies in this area to plant in multiple environments across the varied growing regions of the state, especially in low P soils. In addition to fall nurseries, we would suggest summer nurseries as well. It may also prove helpful to obtain soil samples as well to determine what effect soil P has on P accumulation in various genotypes.

CHAPTER VI

CONCLUSIONS

Although NIRS technology has been utilized since the 1960's, this study looked at the possibility of utilizing a newer instrument, FT-NIRS, to predict maize nutrient components, in particular phosphorus. The advantage with this technology compared to older models is the increased sensitivity of the machine to detect differences in component concentrations.

The focus on phosphorus is critical in today's society from not only an environmental and agricultural standpoint but also in the realm of human nutrition. Plant breeders need a technology that is capable of rapidly analyzing nutrient levels to select the best lines for advancement to meet the nutritional and environmental needs of a growing world population.

This study has shown that calibrations can be developed for the specific FT-NIRS to rapidly analyze phosphorus levels in whole kernel and UDY ground maize samples. Additionally, these same calibrations were used to quantify other composition components, crude protein, fat/oil, and starch. The best practices portion of this research identified the ideal number of scans for each form of maize samples. Selections were made off of which number of scans had the least amount of variation from cup fill and technical replicate. Results indicated that the ideal number of scans was as follows: 128 scans for whole kernel, 96 scans for 1mm ground maize, and 64 scans for 2mm UDY ground maize. The assumption of this study was that ground samples would have less variation from cup fill and technical replicate and more variation from genotype than

whole kernel samples due to homogenization of the sample. Calibration development was facilitated through a diverse sample set in which we found whole kernel calibrations to be less accurate than UDY ground calibrations. After applying pre-processing treatments to the model, we chose PLS_Constant_1D_SG_3-6 as the best combination of treatments for both whole and UDY calibrations. Summary statistics for whole kernel (r = 0.94, PI = 60) and UDY (r = 0.88, PI = 63) models were both similar and promising having high r and PI values. When evaluating the linear models of the calibration and validation samples, both calibrations had high R^2 values (Whole R^2 =0.82, UDY R^2 = 0.74).

The field study produced less than ideal results due to environmental factors beyond our control. High rainfall greatly affected yield and seed quality which may have contributed to higher than expected P levels in maize grain. As a result, there was not enough statistical evidence to validate the calibration models through a field trial.

Our results show that FT-NIRS calibrations can be used to quantify phosphorus levels in maize samples with almost equal success utilizing whole kernel samples versus ground UDY samples. The use of a whole kernel calibration would give plant breeders the ability to rapidly screen lines for advancement. Since we had nearly a two-fold increase between low and high phosphorus levels in our study, this would suggest that lines could be genetically improved for the desired end use of the grain product. However, additional work needs to be conducted including:

1) The implementation of additional field trials in multiple environments and replications with the introduction of more maize lines in the crossing block.

2) The effect of soil P levels on maize grain P accumulation in both temperate and tropical breeding material.

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APPENDIX A

PERCENT VARIATION VALUES FOR BEST PRACTICES STUDY IN WHOLE KERNEL MAIZE

	Crude protein				Phosphorus				Fat				Starch			
16 scans	Cal 1	Cal 2	Cal 3	Cal 4	Cal 1	Cal 2	Cal 3	Cal 4	Cal 1	Cal 2	Cal 3	Cal 4	Cal 1	Cal 2	Cal 3	Cal 4
Genotype	0.0	0.0	0.0	0.0	0.0	22.1	0.0	0.0	0.0	9.7	0.0	0.0	0.0	0.0	0.0	0.0
Cup fill (genotype)	0.0	0.0	50.8	0.0	0.0	0.0	0.9	0.0	24.9	0.0	38.9	4.9	0.0	0.0	0.0	0.0
Rep (cup fill, genotype)	100.0	100.0	49.2	100.0	100.0	77.9	99.1	100.0	75.1	90.3	61.1	95.1	100.0	100.0	100.0	100.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
32 scans																
Genotype	0.4	8.4	0.0	0.0	54.8	84.2	53.1	63.8	55.6	77.3	0.0	54.1	3.1	23.3	16.6	0.3
Cup fill (genotype)	34.0	37.7	66.0	31.1	0.0	4.9	0.3	0.0	0.0	0.2	0.0	0.0	51.6	25.9	36.9	40.7
Rep (cup fill, genotype)	65.6	54.0	34.0	68.9	45.2	10.9	46.7	36.2	44.4	22.5	100.0	45.9	45.3	50.7	46.6	59.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
64 scans																
Genotype	33.5	0.0	0.0	43.5	53.0	81.5	61.5	58.3	67.3	68.9	19.6	51.2	19.0	80.3	8.6	39.1
Cup fill (genotype)	32.4	25.6	45.1	26.5	22.5	12.3	21.6	27.9	0.3	14.9	0.0	0.0	50.0	12.4	51.6	43.6
Rep (cup fill, genotype)	34.1	74.4	54.9	30.0	24.6	6.2	16.9	13.8	32.5	16.2	80.4	48.8	31.0	7.3	39.8	17.3
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
96 scans																
Genotype	0.0	0.0	0.0	0.0	36.0	71.9	47.9	44.9	46.0	76.6	16.7	38.1	0.0	64.6	0.0	3.0
Cup fill (genotype)	49.6	21.8	75.8	51.6	34.1	11.1	30.6	31.7	14.6	7.4	0.0	9.8	24.1	1.4	4.4	38.2
Rep (cup fill, genotype)	50.4	78.2	24.2	48.4	29.9	17.0	21.5	23.4	39.4	16.0	83.3	52.1	75.9	34.0	95.6	58.8
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
128 scans																
Genotype	13.1	0.0	0.0	12.7	73.8	86.7	69.2	68.4	73.6	81.1	35.7	63.3	0.0	70.5	0.0	0.0
Cup fill (genotype)	32.2	68.1	40.0	64.8	9.0	3.6	8.2	14.1	6.7	11.8	0.0	14.5	36.4	11.5	38.2	39.0

Rep (cup fill, genotype)	54.7	31.9	60.0	22.5	17.2	9.8	22.5	17.6	19.7	7.1	64.3	22.2	63.6	18.0	61.8	61.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

APPENDIX B

PERCENT VARIATION VALUES FOR BEST PRACTICES STUDY IN GROUND (2MM) KERNEL MAIZE

	Crude protein			Phosphorus			Fat			Starch		
16 scans	Cal 1	Cal 2	Cal 3	Cal 1	Cal 2	Cal 3	Cal 1	Cal 2	Cal 3	Cal 1	Cal 2	Cal 3
Genotype	90.4	71.0	77.4	56.1	0.0	0.0	96.0	83.8	83.8	2.3	20.4	0.0
Cup fill (genotype)	6.4	8.1	2.9	16.7	14.2	4.3	0.0	3.7	2.0	8.1	0.0	0.0
Rep (cup fill, genotype)	3.2	20.9	19.7	27.3	85.8	95.7	4.0	12.5	14.2	89.7	79.6	100.0
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
32 scans												
Genotype	95.0	84.0	93.8	17.4	0.0	11.7	97.1	96.5	96.4	9.5	0.0	17.1
Cup fill (genotype)	2.4	1.0	4.0	23.1	71.2	40.8	0.0	1.4	1.1	68.7	47.7	42.2
Rep (cup fill, genotype)	2.7	15.1	2.1	59.5	28.8	47.5	3.0	2.1	2.5	21.9	52.3	40.8
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
64 scans												
Genotype	95.2	88.5	95.7	11.0	0.0	53.4	96.6	94.0	95.1	69.0	0.0	35.1
Cup fill (genotype)	3.7	7.5	3.5	11.5	58.1	15.5	2.6	4.6	3.7	3.1	52.7	24.3
Rep (cup fill, genotype)	1.1	4.0	0.9	77.5	41.9	31.0	0.8	1.4	1.2	27.8	47.3	40.6
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
96 scans												
Genotype	95.2	90.6	97.0	30.1	14.6	38.5	95.2	95.8	96.3	88.2	0.0	89.0
Cup fill (genotype)	4.4	6.2	2.8	53.4	74.1	32.0	4.5	3.9	3.4	8.3	70.6	7.6
Rep (cup fill, genotype)	0.4	3.2	0.1	16.6	11.4	29.5	0.3	0.3	0.3	3.5	29.4	3.4
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
128 scans												
Genotype	96.6	88.0	96.6	16.5	35.5	60.3	96.2	97.4	97.0	71.8	0.0	81.9
Cup fill (genotype)	3.3	9.5	3.1	66.5	45.2	9.9	3.8	2.5	2.8	26.6	87.2	14.9

Rep (cup fill, genotype)	0.1	2.5	0.3	17.1	19.4	29.8	0.0	0.1	0.2	1.6	12.8	3.2
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

APPENDIX C

PERCENT VARIATION VALUES FOR BEST PRACTICES STUDY IN GROUND UDY (1MM) KERNEL MAIZE

	Crude protein			Phosphorus			Fat			Starch		
16 scans	Cal 1	Cal 2	Cal 3	Cal 1	Cal 2	Cal 3	Cal 1	Cal 2	Cal 3	Cal 1	Cal 2	Cal 3
Genotype	98.8	90.2	97.2	51.2	20.7	80.2	69.5	95.0	84.5	71.9	46.7	40.5
Cup fill (genotype)	0.3	0.0	2.1	32.0	64.4	4.7	28.2	3.0	10.9	26.7	42.2	50.9
Rep (cup fill, genotype)	0.9	9.8	0.8	16.8	14.9	15.1	2.3	2.1	4.7	1.4	11.1	8.5
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
32 scans												
Genotype	99.7	97.5	98.7	73.2	45.0	89.3	69.8	95.7	85.7	74.8	63.0	54.8
Cup fill (genotype)	0.0	0.4	0.9	24.8	49.1	8.8	28.3	2.8	10.4	24.3	33.5	41.3
Rep (cup fill, genotype)	0.3	2.1	0.4	1.9	5.8	1.9	1.8	1.5	3.9	0.9	3.5	3.9
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
64 scans												
Genotype	99.8	98.7	99.2	72.3	56.1	92.4	68.7	98.3	87.8	74.8	64.4	59.9
Cup fill (genotype)	0.1	1.0	0.7	25.4	42.5	7.1	30.5	1.4	11.5	24.8	34.6	39.7
Rep (cup fill, genotype)	0.1	0.4	0.1	2.4	1.4	0.5	0.9	0.3	0.7	0.4	1.0	0.4
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
96 scans												
Genotype	99.6	98.5	98.6	81.9	66.3	88.8	56.5	97.0	86.3	73.0	56.5	56.8
Cup fill (genotype)	0.0	0.4	1.0	12.2	23.7	4.8	31.3	2.1	6.5	15.5	26.0	31.6
Rep (cup fill, genotype)	0.4	1.1	0.4	5.8	9.9	6.4	12.3	1.0	7.2	11.5	17.5	11.6
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
128 scans												
Genotype	99.4	98.8	97.6	87.4	81.1	95.4	39.8	97.6	78.3	49.9	5.9	25.5
Cup fill (genotype)	0.5	0.8	2.3	11.9	18.5	3.6	59.7	2.1	21.5	49.3	89.2	73.3

Rep (cup fill, genotype)	0.0	0.4	0.0	0.7	0.4	1.0	0.5	0.2	0.2	0.7	5.0	1.3
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

APPENDIX D

WHOLE KERNEL CALIBRATION SAMPLES WITH PEDIGREE INFORMATION AND THE DIFFERENCE BETWEEN ACTUAL AND PREDICTED VALUES FOR INDIVIDUAL COMPONENTS

Calibration sample name	Processing date	Pedigree	Usage	Crude protein diff. x path	Phosphorus diff. x path	Fat diff. x path	Starch diff. x path
1017	5/13/2014	*	Validation	0.37	0	0.05	-1.44
1040	5/13/2014	*	Calibration	-0.17	-0.01	0.01	-0.44
1063	5/13/2014	*	Calibration	0.06	-0.02	-0.26	-2.96
1067	5/13/2014	*	Calibration	-0.07	0	0.66	-2.75
2071	5/13/2014	*	Calibration	-0.39	-0.01	0.03	-1.44
2093	5/13/2014	*	Calibration	-0.09	0	-0.44	-0.75
3005	5/13/2014	*	Calibration	-0.42	-0.02	-0.63	0.9
3013	5/13/2014	*	Calibration	0.01	-0.01	0.24	-1.1
3024	5/13/2014	*	Calibration	0.03	0	0.2	-2.09
3049	5/13/2014	*	Validation	0.36	-0.01	0.39	-1.88
3061	5/13/2014	*	Calibration	-0.15	-0.01	-0.25	0.64
3087	5/13/2014	*	Validation	0.9	0.01	0.46	-2.55
4007	5/13/2014	*	Calibration	0.42	-0.02	0.38	-0.75
4013	5/13/2014	*	Calibration	-0.23	0.02	1.16	-1.75
4047	5/13/2014	*	Calibration	-0.19	0.01	0.97	0.59
4054	5/13/2014	*	Calibration	0.4	0.02	0.98	-1.98
4056	5/13/2014	*	Validation	-1.23	0	0	-1.79
4060	5/13/2014	*	Calibration	-0.48	-0.01	0.62	-0.96
4080	5/13/2014	*	Calibration	-0.18	0	-0.68	0.84
4083	5/13/2014	*	Calibration	-0.36	0.01	0.07	0.91

11SMPSA734101	12/7/2011	CML 161 Q3 X TX812 Q11	Calibration	0.39	0	0.41	0.28
11SMPSA734701	12/7/2011	DKC68-06	Calibration	0.08	0.01	0.22	0.97
11SMPSA737301	12/7/2011	Hallauer1 Q1 X TX813 Q12	Calibration	0.08	0	1	1.26
11SMPSA738901	12/7/2011	Tx 814 Q13 X LH287 Q7	Calibration	0.41	0.02	0.7	-2.18
11SMPSA740601	12/7/2011	CML 161 Q3 X TX812 Q11	Calibration	0.6	0.01	0.43	0.67
11SMPSA741701	12/7/2011	DKC68-06	Calibration	0.4	0	0.45	-1.82
11SMPSA741801	12/7/2011	Tx 813 Q12 X CML 161 Q3	Calibration	0.16	0.01	1.43	-0.41
11SMPSA744201	12/7/2011	Tx 814 Q13 X CML176 Q5	Calibration	0.23	-0.02	-0.74	1.04
C12-RJW-8-41	5/13/2014	*	Validation	-1	0.01	0.28	3.2
CC10-GO-011	5/9/2011	((B104-1xTx714-B/B110xFR2128-B)-12-4-B-B-B-B/SCR82-B)-B-B-1-B-B-B/LH195/LH195	Calibration	0.32	0	-0.42	1.07
CC10-GO-046	5/9/2011	(CML379/CML311-B-1-B-B-B-B/Tx110)-B-1-B-1-B/LH195	Validation	0.01	0.03	-0.33	-0.27
CC10-GO-064	5/9/2011	ArgnetineFlintyComposite-C(1)-26-B-B/LH195	Calibration	-0.17	0	0.11	-0.66
CC10-GO-070	5/9/2011	(CML450-B/Tx110)-B-3-B-3-B/LH287RR2	Calibration	0.08	0.01	-0.4	-0.58
CC10-GO-071	5/9/2011	ArgnetineFlintyComposite-C(1)-7-B-B/LH287RR2	Calibration	-0.2	0	0.29	1.44
CC10-GO-084	5/9/2011	(Bs13(S)C8-26-1-BxNC380)-B-B-B-B-B/LH287	Calibration	0.05	0.01	-0.23	-0.72
CC10-GO-120	2/4/2011	ArgnetineFlintyComposite-C(1)-29-B-B/LH287RR2	Calibration	-0.2	0	-1.48	0.67
CC10-GO-126	2/4/2011	W4700	Calibration	-0.24	0.01	-0.96	0.88
CC10-GO-171	5/9/2011	((NC300/Tx772)-B-1-B2-B-B-B/CML161)-B-B-1-B-B-B/LH195	Calibration	-0.01	0.02	0.47	-0.3
CC10-GO-191	5/9/2011	((B104-1xTx714-B/B110xFR2128-B)-12-4-B-B-B-B/SCR82-B)-B-B-1-B-B-B2/LH195	Calibration	0.14	0.01	-0.51	-0.98
CC10-GO-211	2/4/2011	(\CML450-B/Tx110)-B-1-B-1-B/LH195	Validation	-0.62	0.01	0.05	1.77
CC10-GO-326	2/4/2011	ArgnetineFlintyComposite-C(1)-7-B-B/LH287RR2	Calibration	-0.01	-0.02	-0.73	0.28
CC10-GO-330	5/9/2011	(LAMA2002-25-5-B/LAMA2002-2-3-B)-B-B-1-1-B/LH287	Validation	-0.01	-0.02	-0.24	-2.03
CC10-GO-351	5/9/2011	ArgnetineFlintyComposite-C(1)-36-B2-B1/LH195	Calibration	-0.09	0	-0.01	0.88
CC10-GO-382	5/9/2011	(BS13(S)C8-33-1-B-B-BxTx745)-B-B-B-B-B/LH287	Validation	0.28	0	-0.06	1.6

CC10-GO-386	5/9/2011	WE06-6001ArgentineComposite	Validation	-0.12	0.03	0.09	1.71
CS06-1018Y-11	5/9/2011	*	Calibration	0.15	0.03	1.45	-2.39
CS06-1018Y-65	5/9/2011	*	Calibration	0.04	0.01	0.37	-1.24
CS07-1010-11	5/9/2011	*	Calibration	-0.01	-0.03	-0.36	-0.53
CS07-1042-1	5/9/2011	*	Calibration	-0.51	0	1	-0.96
CS10-AMS-040	5/9/2011	*	Calibration	0.05	0	-1.15	-2.09
CS10-ARG1-01	5/9/2011	Argnetine Flinty Composite-C(1)-57-B1-B/LH195	Calibration	0.01	0	-0.37	-1.34
CS10-ARG1-15	5/9/2011	Argnetine Flinty Composite-C(1)-6-B1-B/LH195	Validation	-0.94	-0.01	-0.64	1.94
CS10-ARG1-18	5/9/2011	Argnetine Flinty Composite-C(1)-5-B-B/LH287RR2	Calibration	0.01	0.01	-0.24	1.22
CS10-ARG1-27	5/9/2011	Argnetine Flinty Composite-C(1)-20-B-B/LH287RR2	Calibration	0.55	-0.01	0.03	-0.03
CS10-ARG1-33	5/9/2011	Argnetine Flinty Composite-C(1)-36-B2-B1/LH195	Validation	0.39	-0.03	-0.77	1.57
CS10-ARG1-55	5/9/2011	Argnetine Flinty Composite-C(1)-22-B-B2/LH195	Calibration	-0.1	-0.02	-0.42	3.24
CS10-ARG1-57	5/9/2011	Argnetine Flinty Composite-C(1)-16-B-B/LH195	Calibration	0.07	0.02	-0.94	1.11
CS10-ARG1-80	5/9/2011	Argnetine Flinty Composite-C(1)-6-B1-B/LH195	Validation	-0.06	0.02	0.09	0.78
CS10-CTP-128	5/9/2011	Belle BXCO28VT3	Validation	0.2	-0.01	0.67	-1.7
CS10-EGT2-098	5/9/2011	((CML 326/Tx772)-B-11-B-B-B-B/CML161)-B-B-2-B-B-B/LH195	Calibration	0.32	-0.01	-0.19	-0.7
CS10-QPMX-026	5/9/2011	P31G66(2007)	Validation	1.05	0.02	-0.51	0.27
CS10-QPMX-112	5/9/2011	CML269/TX130-B-B-B-1-1-B-B-B-B-B-B-B/X "WHT QPM TSTR"	Calibration	-0.02	0	-0.72	-0.05
CS10-QPMX-186	5/9/2011	(LAMA2002-10-1-B/BS13(S)C8-34-1-B-B-B-B-B)-B-B-1-1-B-B-B/X "WHT QPM TSTR"	Calibration	-0.12	0.01	-0.52	-2.16
CS10-QPMX-205	5/9/2011	Red Ear 2-2-2-1-1-2-B/X "WHT QPM TSTR"	Calibration	-0.37	-0.02	-0.82	-0.24
CS10-SERAT-01	2/4/2011	Mp313E x Mp317	Calibration	-0.21	0	-0.36	1.07
CS10-SERAT-08	2/4/2011	LB08Iso:8078	Calibration	-0.1	-0.01	-0.27	2.17
CS10-SERAT-31	2/4/2011	Mp494 x Mp717	Calibration	-0.09	0	1.07	0.12
CS10-SERAT-33	2/4/2011	CS09-QPMX-050	Calibration	0.2	0.03	1.15	1.59
CS10-SERAT-38	2/4/2011	WE-ISO-Pro-064	Calibration	-0.22	0	0.37	0.86

CS10-SERAT-61	2/4/2011	Pioneer 31D58	Calibration	0.28	0	0.14	-0.87
CS10-SERAT-71	2/4/2011	NC300 x Mp715	Calibration	0.04	0.01	-0.09	1.37
CS10-SERAT-74	2/4/2011	Mp313E x Mp496	Calibration	-0.54	-0.02	0.09	-0.71
CS10-SERAT-86	2/4/2011	CS09-QPMX-005	Calibration	-0.16	0	0.39	0.05
CS10-SERAT-89	2/4/2011	LB08Iso:6059	Calibration	0.14	0.03	0.28	1.11
CS10-USDA-029	2/4/2011	*	Calibration	0	-0.01	0.5	-0.83
CS10-USDA-126	2/4/2011	*	Calibration	0.59	0.02	-0.36	0.65
CS10-USDA-224	2/4/2011	*	Calibration	-0.81	0	0.08	-0.03
CS10-USDA-320	2/4/2011	*	Calibration	-0.43	0	-0.26	-0.61
CS10-USDA-406	2/4/2011	*	Calibration	0.15	0.02	-0.52	-1.3
CS10-USDA-407	2/4/2011	*	Calibration	0.39	0.01	-0.14	-1.54
CS10-USDA-410	2/4/2011	*	Calibration	-0.25	0.02	-0.31	0.6
CS10-USDA-418	2/4/2011	*	Calibration	-0.26	0.02	-0.08	-0.94
CS10-USDA-422	2/4/2011	*	Calibration	-0.36	-0.01	-1.03	-2.04
CS10-USDA-649	2/4/2011	*	Calibration	0.94	0.01	0.51	-1.09
CS10-USDA-679	2/4/2011	*	Calibration	-0.38	-0.01	-1.3	1.14
CS10-USDA-725	2/4/2011	*	Calibration	-0.34	0	0.2	0.7
CS10-USDA-743	2/4/2011	*	Calibration	0.38	0.03	0.89	-0.94
CS10-USDA-765	2/4/2011	*	Calibration	-0.05	-0.02	-0.02	0.71
CS10-USDA-816	2/4/2011	*	Calibration	-0.24	-0.01	-0.13	-2.53
CS10-USDA-835	2/4/2011	*	Calibration	0.25	-0.02	-0.19	0.83
CS10-USDA-862	2/4/2011	*	Calibration	-0.07	0.02	0.55	-0.14
CS10-USDA-880	2/4/2011	*	Calibration	-0.12	-0.01	0.17	0.38
CS10-WHTT-009	5/9/2011	((Tx114 (B73w)-B x CML343/Tx110 x Pop24)-B-B-B-4-B-B-B-B/CML78)-B-2-B-2-B/LH195	Validation	-0.28	-0.02	-1.01	-1.21
CS10-WHTT-012	5/9/2011	(CML379/CML311-B-1-B-B-B-B/Tx110)-B-1-B-2-B/LH287RR2	Calibration	0	-0.02	0.14	0.6
CS10-WHTT-029	5/9/2011	(CML450-B/Tx110)-B-2-B-2-B/LH287RR2	Validation	0.83	0.04	-0.75	-2.42

CS10-WHTT-072	5/9/2011	W08-LH287-131	Calibration	-0.53	0.01	-0.51	0.18
CS10-WHTT-096	5/9/2011	(\CML450-B/Tx110)-B-1-B-1-B/LH195	Calibration	-0.49	-0.01	-0.73	1.57
CS10-WHTT-114	5/9/2011	(CML379/CML311-B-1-B-B-B-B/Tx110)-B-1-B-2-B/LH195	Calibration	-0.09	-0.02	-0.8	-0.88
CS11-AMQY-120	12/7/2011	Tx 811 Q10 X CML 161 Q3	Calibration	0.16	-0.01	-0.08	-1.91
CS11-AMQY-163	12/7/2011	Tx 812 Q11 X LH195 Q6	Calibration	0.34	-0.01	-0.24	0.46
CS11-AMQY-202	12/7/2011	Tx 812 Q11 X Hallauer1 Q1	Calibration	-0.66	0.01	0.43	0.36
CS12-DYTL-101A	1/3/2016	AMATLCOHS71-1-1-2-1-1-1-BBBB-B-B-B	Calibration	-0.27	-0.02	0.07	1.3
CS12-DYTL-146A	1/3/2016	B64	Calibration	-0.49	-0.04	0.08	2.23
CS12-DYTL-221A	1/3/2016	Ki3	Calibration	-0.25	-0.03	-0.29	1.93
CS12-DYTL-228A	1/3/2016	Pa91	Calibration	-0.46	-0.01	-0.33	0.8
CS12-DYTL-234A	1/3/2016	Mt42	Calibration	-0.27	0.03	0.86	4.09
CS12-DYTL-240A	1/3/2016	H99	Validation	0.1	-0.04	-0.59	2.41
CS12-DYTL-242A	1/3/2016	CO255	Validation	-0.86	0.01	0.39	-0.41
CS12-DYTL-258A	1/3/2016	NC368	Validation	-0.26	0	-0.18	-1.46
CS12-DYTL-294A	1/3/2016	I137TN	Calibration	-0.47	0.01	0.51	-3.43
CS12-DYTL-334A	1/3/2016	A682	Validation	0.08	0.03	-0.02	-0.1
CS12-DYTL-376A	1/3/2016	CML158Q	Calibration	-0.04	-0.01	0.27	-1.45
CS12-DYTL-437A	1/3/2016	Mp07:153	Calibration	0.2	0.03	0.59	-2.9
CS12-DYTL-596A	1/3/2016	P39Goodman-Buckler	Calibration	-0.63	-0.05	-0.54	0.84
CS12-DYTL-641A	1/3/2016	38-11	Validation	-0.48	0.02	-0.07	-1.95
CS12-DYTL-673A	1/3/2016	NC330	Validation	1.65	0.01	-1.15	-3.95
CS12-DYTL-680A	1/3/2016	CO255	Calibration	0.18	0.03	0.63	2.77
CS12-DYTL-754A	1/3/2016	Ki43	Validation	0.06	0.04	0	5.32
CS12-DYTL-782A	1/3/2016	Ki3	Calibration	-0.11	0	-0.35	5.46
CS12-DYTL-783A	1/3/2016	GT112	Calibration	0.1	0.02	0.34	5.79
CS12-DYTL-794A	1/3/2016	[MBRC6BcG395-1-B-#-2-2-B-B-B-B-B/CML312SR]-1-1	Calibration	-0.19	0	-0.48	8.45

CS12-DYTL-910A	1/3/2016	CML77	Calibration	0.55	0.04	-0.61	0.49
CS12-LINC-036-B11	5/13/2014	Tx772	Calibration	-0.33	0	0.63	0.79
CS12-LINC-040-B22	5/13/2014	Tx903 - (Lfy2361-B/Tx114(B73w)-B Dark blue-B)Tx114/Lfy2304-B-B-B-1-3-B-B-B-3-B-B-B22	Calibration	-0.14	0	0.17	1.58
CS12-LIYT-111A	1/3/2016	CMV3	Calibration	-0.35	-0.03	-0.42	4.01
CS12-LIYT-125A	1/3/2016	H95	Validation	0.21	0.01	-0.06	1.35
CS12-LIYT-150A	1/3/2016	Pa91	Calibration	0.36	0.03	0.32	-0.65
CS12-LIYT-162A	1/3/2016	Oh603	Validation	-0.35	0.01	-0.04	-0.37
CS12-LIYT-185A	1/3/2016	NC264	Calibration	-0.19	-0.01	-0.4	-0.05
CS12-LIYT-305A	1/3/2016	Mo47	Calibration	0.12	0	-0.84	1.12
CS12-LIYT-532A	1/3/2016	NC322	Calibration	-0.85	-0.01	-0.99	2.79
CS12-LIYT-547A	1/3/2016	Ki43	Validation	-1.3	-0.06	-0.74	5.14
CS12-LIYT-745A	1/3/2016	NC358	Calibration	-0.11	-0.01	0.13	0.15
CS12-LIYT-752A	1/3/2016	GT112	Calibration	-0.29	-0.02	-0.64	-1.28
CS12-RJW-10-414	5/13/2014	*	Calibration	0.07	-0.04	-0.36	-2.96
CS12-RJW-10-428	5/13/2014	*	Validation	0.39	0	-0.21	1.69
CS12-RJW-10-573	5/13/2014	*	Validation	1.04	0.03	-1.04	-2.91
CS12-RJW-10-647	5/13/2014	*	Calibration	-0.33	-0.02	-0.48	1.46
CS12-RJW-161-1	5/13/2014	*	Calibration	-0.35	-0.02	-1.03	1.91
CS12-RJW-165-1	5/13/2014	*	Calibration	0.02	-0.01	0.07	1.11
CS12-RJW-250-1	5/13/2014	*	Calibration	0.58	0.02	0.84	-1.75
CS12-RJW-261-1	5/13/2014	*	Calibration	-0.19	-0.04	-0.15	0.65
CS12-RJW-390-1	5/13/2014	*	Calibration	0.19	-0.01	-0.5	1.27
CS12-RJW-527-1	5/13/2014	*	Calibration	0.24	0.01	0.51	-2.55
CS12-RJW-8-40	5/13/2014	*	Calibration	0.81	0	-0.65	0.55
CS12-RJW-8-51	5/13/2014	*	Calibration	-0.58	0	0.89	-0.29
CS12-RJW-9-220	5/13/2014	*	Calibration	-0.66	0	-0.35	1.15
CS12-RJW-9-236	5/13/2014	*	Validation	1.58	0.04	1.19	-1.83

CS13-DOTH-098-B9	5/13/2014	(((RedHybridEar-B-1-2-2-1-B)//(Lfy2361-B/Tx114(B73w)-BDarkblue-B)Tx114/Lfy2304-B-B-B-1-2-B-B-B-2-B)///((RedEar5-2-4-1-1-2)/(Ethiopia15-B-5-1-B-B2-B-1-B-B)))-5#-1-B4-B4-B9	Calibration	-0.04	-0.01	-0.27	-0.25
CS13-DOTH-102-B2	5/13/2014	4 way cross progeny (Tx772, B73 Oleic, Tx903, Tx906)	Calibration	0.35	0.01	0.83	-3.2
CS13-EOTH-21-B10	5/13/2014	87916'	Calibration	0.05	0.01	0.85	-0.54
CS13-EOTH-52-B4	5/13/2014	(((PB80/(Ethiopia15-B-5-1-B-B2-B-1-B-B)-B4#-2)//(PB80/(Ethiopia15-B-5-1-B-B2-B-1-B-B)-B4#-1))-B5//HopiBlue)-B7-B4	Calibration	0.78	0	0.26	-1.37
CS13-INC-020-B18	5/13/2014	LAMA2002-58-3-B-B-B-B-B-B-1-B19-B18	Calibration	0.18	0.01	0.76	-0.03
CS13-OTH-055-X-OTH-049	5/13/2014	((((LH123HT/(RedEar5-2-4-1-1-2))-1-B4-1#)//LH123HT)-B2///LH123HT)	Calibration	0.21	0.04	0.96	-0.28
CS13-OTH-114-B2	5/13/2014	(PHG84/WC1082)-B3-6-B5	Calibration	0.38	0	0.38	1.26
CS14-SERAT-005	2/18/2015	((Tx740/Mp715)//(Tx772/Mp313))-#/((Tx772/Mp715)//(Tx740/Mp313E))-#	Calibration	0.05	0.01	0.01	0.35
CS14-SERAT021	2/18/2015	Tx-WX-9	Calibration	0.34	0.03	-0.83	1.14
CS14-SERAT-46	2/18/2015	Tx-WX-8	Validation	0.73	0.03	-0.19	-2.14
CS14-SERAT-51	2/18/2015	Mp13:9025 x Mp13:9026	Calibration	-0.08	0	-0.13	0.89
CS14-SERAT-70	2/18/2015	((Tx740/Mp715)//(Tx772/Mp313))-#/((Tx772/Mp715)//(Tx740/Mp313E))-#	Calibration	0.39	-0.01	-0.72	-1.65
CS14-SERAT-75	2/18/2015	Mp13:9035 x Mp13:9036	Calibration	-0.49	-0.01	-1.36	0.04
CS14-SERAT-81	2/18/2015	Hi63 x NC466	Calibration	-0.26	0.03	-0.08	-2.21
CS14-SERAT-104	2/18/2015	SS1\X\(LAMA2002-35-2-B-B-B-B/CG44)-1-3-B-B14-B10	Calibration	0.65	0.03	-0.24	-0.87
CS14-SERAT-106	2/18/2015	GRACE E-5 (E-1) x DK888	Validation	-0.44	0	-0.36	-1.52
CS14-SERAT-108	2/18/2015	Tx-WX-8	Calibration	0.11	0	-0.32	-2.22
CS14-SERAT-109-OP10	2/18/2015	*	Validation	0.55	0	0.37	-1.49
CS15-NILAS-8047	1/3/2016	*	Validation	-0.77	0.01	0.81	-1.53
CS15-NILAS-8049	1/3/2016	*	Calibration	0.06	0.02	0.74	-0.25
CS15-NILAS-8054	1/3/2016	*	Validation	-0.47	0.05	0.52	-0.91
CS15-NILAS-8081	1/3/2016	*	Calibration	0.8	0.03	0.92	-3.39

CS15-NILAS-8145	1/3/2016	*	Calibration	0.55	0.04	-0.82	1.8
CS15-NILAS-8157	1/3/2016	*	Validation	1.29	0.05	-0.54	3.5
CS15-NILAS-8168	1/3/2016	*	Calibration	1.11	0.02	0.19	3.39
CS15-NILAS-8323	1/3/2016	*	Calibration	0.33	0.04	0.65	-2.53
CS15-NILAS-8368	1/3/2016	*	Calibration	-0.82	-0.02	-1.78	5.92
CS15-NILAS-8900	1/3/2016	*	Calibration	0.22	0.02	0.6	-4.7
IA15-NILAS-1621	1/3/2016	*	Calibration	-0.7	-0.02	0.35	-0.05
IA15-NILAS-1637	1/3/2016	*	Calibration	-0.32	-0.03	0.06	-2.36
IA15-NILAS-1642	1/3/2016	*	Calibration	0.43	0.03	0.87	1.15
IA15-NILAS-1672	1/3/2016	*	Calibration	-0.01	-0.01	0.33	1.42
IA15-NILAS-1961	1/3/2016	*	Calibration	-0.11	0	-0.3	3.5
IA15-NILAS-2075	1/3/2016	*	Validation	0.45	0.01	0.36	-4.2
IA15-NILAS-2100	1/3/2016	*	Validation	0.14	-0.03	0.69	-2.9
IA15-NILAS-2584	1/3/2016	*	Calibration	0.35	-0.03	-0.25	-1.63
IA15-NILAS-2681	1/3/2016	*	Calibration	0.24	0.02	0.54	-1.63
IA15-NILAS-2725	1/3/2016	*	Validation	0.67	0.01	0.42	-4.21
IA15-NILAS-2944	1/3/2016	*	Calibration	0.44	0.02	1.27	-1.82
IA15-NILAS-2962	1/3/2016	*	Calibration	0.66	-0.01	0.35	-0.89
IA15-NILAS-2992	1/3/2016	*	Calibration	0.33	0.01	0.54	-2.92
IA15-NILAS-3043	1/3/2016	*	Calibration	0.07	-0.02	-0.55	-1.5
IA15-NILAS-3046	1/3/2016	*	Calibration	0.05	-0.02	0.11	-3.8
IA15-NILAS-3078	1/3/2016	*	Calibration	0.68	0	0.33	-2.32
IA15-NILAS-3122	1/3/2016	*	Calibration	0.23	-0.01	-0.13	0.77
TH10-TAC-101	2/4/2011	TAC-5	Calibration	-0.27	-0.01	0.44	0.49
TH10-TAC-145	2/4/2011	TAC-8	Validation	0.28	0.03	-0.02	0.46
TH10-TAC-221	2/4/2011	TAC-5	Calibration	0.68	0	-0.19	0.42
TH10-TAC-319	2/4/2011	TAC-8	Validation	-0.17	0.01	0.38	1.68

TH10-TAC-336	2/4/2011	TAC-5	Validation	-0.13	0.05	0.81	2.42
TH10-TAC-401	2/4/2011	TAC-8	Validation	0.69	0.05	0.65	0.89
TH10-TAC-441	2/4/2011	TAC-5	Calibration	-0.01	0	0.53	1.13
WE10-AMS-220	5/9/2011	*	Calibration	-0.26	-0.02	0.32	-0.24
WE10-ARG1-25	5/9/2011	ArgnetineFlintyComposite-C(1)-14-B-B/LH287RR2	Calibration	-0.26	0	0.75	-1.18
WE10-ARG1-48	5/9/2011	ArgnetineFlintyComposite-C(1)-3-B-B/LH287RR2	Validation	0.07	0.02	0.69	-2.78
WE10-QPMX-033	5/9/2011	Pop.69TempladoAmarilloQPM-B-B-B2-12-B-B-B-B-B-B-B-1/X_WHTQPMTSTR	Validation	-0.91	-0.06	-0.77	0.74
WE10-QPMX-035	5/9/2011	(B97xCML326-B/Tx770xA645)-2-2-B-B-B-B-B-B-B-B//X_WHTQPMTSTR	Validation	-0.57	0.03	0.6	0.31
WE10-QPMX-094	5/9/2011	P31G66(2007)	Validation	0.65	-0.05	-0.47	0.32
WE10-QPMX-132	5/9/2011	(LAMA2002-12-1-B/(CML325/B104)-B-1-B-B-B-B)-B-B2-4-2-B-B-B//X_WHTQPMTSTR	Calibration	-0.75	-0.02	-0.74	0.51
WE10-QPMX-196	5/9/2011	Tx804//X_WHTQPMTSTR	Calibration	-0.11	0	-0.86	1.66
WE10-WHTT-011	5/9/2011	((Tx114(B73w)-BxCML343/Tx110xPop24)-B-B-B-4-B-B-B-B/CML78)-B-2-B-1-B/LH287RR2	Calibration	0.11	0	0.28	0.1
WE10-WHTT-091	5/9/2011	((CML373/FR825)/(CML269/Tx110)-1-B-B-B-B/CML269/TX114-B-B-B-1-1-B-B-B)-B-1-B-3-B/LH195	Validation	0.22	0.01	-0.54	-0.88
WE10-WHTT-105	5/9/2011	((CML373/FR825)/(CML269/Tx110)-1-B-B-B-B/CML269/TX114-B-B-B-1-1-B-B-B-B-B)-B-1-B-3-B/LH195	Validation	0.99	0	-1.18	1.13
WE11-AMQY-159	12/7/2011	Tx 812 Q11 X LH195 Q6	Calibration	-0.02	0.02	-0.72	-0.6
WE11-AMQY-215	12/7/2011	DKC68-06	Calibration	0.44	0.02	-0.03	-1.77
WE11-AMQY-267	12/7/2011	Tx 810 Q9 X CML 176 Q5	Calibration	-0.36	-0.05	-1.13	2.19

APPENDIX E

UDY GRIND CALIBRATION SAMPLES WITH PEDIGREE INFORMATION AND THE DIFFERENCE BETWEEN ACTUAL AND PREDICTED VALUES FOR INDIVIDUAL COMPONENTS

Calibration sample name	Processing date	Pedigree	Usage	Crude Protein diff. x path	Phosphorus diff. x path	Fat diff. x path	Starch diff. x path
1017	5/13/2014	*	Validation	0.03	-0.03	0.08	-3.29
1040	5/13/2014	*	Calibration	0.29	0.01	-0.15	-3
1041	5/13/2014	*	Validation	0.12	-0.04	-0.02	-2.43
1058	5/13/2014	*	Validation	0.26	0.02	0.04	-1.91
1061	5/13/2014	*	Validation	0.26	0	0.17	-1.62
1063	5/13/2014	*	Validation	0.13	0	-0.02	-4.53
1067	5/13/2014	*	Calibration	0.34	0.01	0.09	-3.87
2071	5/13/2014	*	Validation	-0.17	0.03	-0.16	-0.93
2093	5/13/2014	*	Calibration	-0.29	0	-0.22	-1.32
3005	5/13/2014	*	Validation	-0.1	0.01	-0.07	-1.03
3013	5/13/2014	*	Calibration	-0.34	-0.02	0.33	-1.33
3024	5/13/2014	*	Validation	-0.05	0.03	0.09	-2.02
3049	5/13/2014	*	Calibration	0.47	-0.01	0.03	-3.22
3061	5/13/2014	*	Calibration	-0.09	0.03	-0.06	-0.96
3087	5/13/2014	*	Calibration	0.07	-0.01	0.29	-1.82
4007	5/13/2014	*	Calibration	0.29	0.01	0.13	0.63
4013	5/13/2014	*	Calibration	-0.11	0.02	0.51	-0.92
4047	5/13/2014	*	Calibration	-0.1	0.01	0.33	1.55
4054	5/13/2014	*	Calibration	0.04	0.01	0.36	-0.13
4056	5/13/2014	*	Calibration	-0.2	0.01	-0.05	-3.1

4060	5/13/2014	*	Validation	0	0	0.12	-0.22
4083	5/13/2014	*	Calibration	0.15	0.02	-0.01	0.24
11SMPSA734101	12/7/2011	CML 161 Q3 X TX812 Q11	Calibration	-0.54	0.01	0.22	1.63
11SMPSA738901	12/7/2011	Tx 814 Q13 X LH287 Q7	Validation	0.22	0.02	0.07	1.96
11SMPSA740601	12/7/2011	CML 161 Q3 X TX812 Q11	Calibration	-0.32	-0.01	0.16	2.44
11SMPSA741701	12/7/2011	DKC68-06	Calibration	0.4	0.02	0	0.92
11SMPSA741801	12/7/2011	Tx 813 Q12 X CML 161 Q3	Calibration	-0.09	-0.01	0.33	1.12
11SMPSA744201	12/7/2011	Tx 814 Q13 X CML176 Q5	Calibration	-0.01	-0.02	0.26	0.7
129-64	2/18/2015	*	Calibration	0.25	0	-0.51	-1.05
203-84	2/18/2015	*	Calibration	-0.24	0.03	-0.07	-0.87
226-20	2/18/2015	*	Validation	0.04	0.01	-0.65	-0.57
306-93	2/18/2015	*	Validation	-0.3	-0.03	-0.33	-0.77
331-33	2/18/2015	*	Validation	0.1	0.03	-0.37	-1.63
334-25	2/18/2015	*	Calibration	-0.16	-0.03	-0.32	0.27
350-20	2/18/2015	*	Calibration	-0.27	0	-0.53	-1.17
352-74	2/18/2015	*	Calibration	-0.44	0.01	-0.31	-2.73
46-57	2/18/2015	*	Calibration	0.38	0.06	-0.07	-0.4
92-57	2/18/2015	*	Calibration	-0.04	0.03	-0.26	-0.97
CC10-GO-011	5/9/2011	((B104-1xTx714-B/B110xFR2128-B)-12-4-B-B-B-B/SCR82-B)-B-B-1-B-B-B/LH195/LH195	Calibration	0.05	0.04	0.25	-0.14
CC10-GO-046	5/9/2011	(CML379/CML311-B-1-B-B-B-B/Tx110)-B-1-B-1-B/LH195	Calibration	0.13	0.01	0	-1.11
CC10-GO-064	5/9/2011	ArgnetineFlintyComposite-C(1)-26-B-B/LH195	Calibration	0.27	0.05	0.47	-0.77
CC10-GO-070	5/9/2011	(CML450-B/Tx110)-B-3-B-3-B/LH287RR2	Validation	0.05	0.02	-0.1	-1.47
CC10-GO-071	5/9/2011	ArgnetineFlintyComposite-C(1)-7-B-B/LH287RR2	Validation	-0.04	0.06	0.16	-0.54
CC10-GO-084	5/9/2011	(Bs13(S)C8-26-1-BxNC380)-B-B-B-B-B/LH287	Calibration	-0.04	0.02	-0.26	-1.45

CC10-GO-120	2/4/2011	ArgnetineFlintyComposite-C(1)-29-B-B/LH287RR2	Validation	-0.1	0.07	-0.06	0.68
CC10-GO-126	2/4/2011	W4700	Calibration	-0.4	0.01	-0.34	0.52
CC10-GO-191	5/9/2011	((B104-1xTx714-B/B110xFR2128-B)-12-4-B-B-B-B/SCR82-B)-B-B-1-B-B-B2/LH195	Calibration	-0.32	0.03	0.02	-0.77
CC10-GO-211	2/4/2011	(\CML450-B/Tx110)-B-1-B-1-B/LH195	Calibration	-0.25	0.06	-0.1	3.03
CC10-GO-305		*	Calibration	0.03	-0.01	-0.78	1.21
CC10-GO-326	2/4/2011	ArgnetineFlintyComposite-C(1)-7-B-B/LH287RR2	Validation	-0.33	0.01	-0.1	1.42
CC10-GO-330	5/9/2011	(LAMA2002-25-5-B/LAMA2002-2-3-B)-B-B-1-1-B/LH287	Calibration	-0.2	-0.02	-0.17	-1.08
CC10-GO-348		(CML379/CML311-B-1-B-B-B-B/Tx110)-B-2-B-4-B/LH195	Calibration	-0.01	-0.02	-0.47	-0.22
CC10-GO-351	5/9/2011	ArgnetineFlintyComposite-C(1)-36-B2-B1/LH195	Validation	-0.08	0.01	-0.07	0.35
CC10-GO-382	5/9/2011	(BS13(S)C8-33-1-B-B-BxTx745)-B-B-B-B-B/LH287	Calibration	0.28	0	0.38	1.66
CC10-GO-386	5/9/2011	WE06-6001ArgentineComposite	Calibration	-0.17	0.01	0.16	2.05
CS06-1018Y-11	5/9/2011	*	Validation	-0.31	0.02	1.33	-1.85
CS07-1010-11	5/9/2011	*	Calibration	-0.09	-0.06	-0.13	0.43
CS07-1042-1	5/9/2011	*	Calibration	0.04	-0.02	1.01	-1.04
CS10-AMS-040	5/9/2011	*	Calibration	0.12	-0.04	0.25	0.4
CS10-ARG1-15	5/9/2011	Argnetine Flinty Composite-C(1)-6-B1-B/LH195	Calibration	-0.23	-0.02	0.18	1
CS10-ARG1-18	5/9/2011	Argnetine Flinty Composite-C(1)-5-B-B/LH287RR2	Validation	0.02	0.02	0.15	2.57
CS10-ARG1-27	5/9/2011	Argnetine Flinty Composite-C(1)-20-B-B/LH287RR2	Calibration	-0.15	0.02	0.57	0.4
CS10-ARG1-33	5/9/2011	Argnetine Flinty Composite-C(1)-36-B2-B1/LH195	Calibration	0.17	0.02	0.36	0.28
CS10-ARG1-57	5/9/2011	Argnetine Flinty Composite-C(1)-16-B-B/LH195	Calibration	-0.13	0.01	0.43	0.81
CS10-ARG1-80	5/9/2011	Argnetine Flinty Composite-C(1)-6-B1-B/LH195	Validation	-0.16	0.03	0.35	-1.37
CS10-CTP-128	5/9/2011	Belle BXCO28VT3	Calibration	-0.03	-0.01	0.7	0.78

CS10-EGT2-098	5/9/2011	((CML 326/Tx772)-B-11-B-B-B-B/CML161)-B-B-2-B-B-B/LH195	Calibration	-0.12	-0.05	0.13	1.57
CS10-QPMX-026	5/9/2011	P31G66(2007)	Calibration	0.24	-0.01	-0.33	0.76
CS10-QPMX-186		(LAMA2002-10-1-B/BS13(S)C8-34-1-B-B-B-B-B)-B-B-1-1-B-B-B/X "WHT QPM TSTR"	Validation	-0.08	0	0.18	-1.48
CS10-QPMX-205	5/9/2011	(LAMA2002-10-1-B/BS13(S)C8-34-1-B-B-B-B-B)-B-B-1-1-B-B-B/X "WHT QPM TSTR"	Calibration	-0.07	0.02	0.06	-1.97
CS10-SERAT-08	5/9/2011	Red Ear 2-2-2-1-1-2-B/X "WHT QPM TSTR"	Calibration	-0.2	-0.01	-0.14	1.58
CS10-SERAT-31	2/4/2011	Mp494 x Mp717	Validation	0.14	0.02	0.16	-0.16
CS10-SERAT-33	2/4/2011	CS09-QPMX-050	Calibration	0.07	0.01	0.3	-1.28
CS10-SERAT-38	2/4/2011	WE-ISO-Pro-064	Validation	0.24	-0.01	-0.26	1.56
CS10-SERAT-61	2/4/2011	Pioneer 31D58	Calibration	0	0.01	0.12	-0.14
CS10-SERAT-89	2/4/2011	LB08Iso:6059	Calibration	0.02	0.05	-0.15	0.16
CS10-USDA-029	2/4/2011	*	Calibration	0.29	0	-0.31	-1.3
CS10-USDA-126	2/4/2011	*	Calibration	0.47	-0.01	-0.23	1.35
CS10-USDA-166	2/4/2011	*	Validation	0.35	0.02	-0.08	-0.19
CS10-USDA-224	2/4/2011	*	Calibration	-0.55	0.01	-0.04	-0.92
CS10-USDA-244	2/4/2011	*	Validation	0.25	-0.04	-0.06	0.93
CS10-USDA-320	2/4/2011	*	Calibration	-0.05	0	0.46	-1.41
CS10-USDA-406	2/4/2011	*	Calibration	0.07	-0.01	-0.3	0.66
CS10-USDA-407	2/4/2011	*	Calibration	0.58	-0.02	-0.09	-0.11
CS10-USDA-418	2/4/2011	*	Calibration	0.06	-0.03	0.57	-2.41
CS10-USDA-422	2/4/2011	*	Calibration	0.02	0.02	0.59	-1.12
CS10-USDA-649	2/4/2011	*	Calibration	0.68	0	0.16	-0.55
CS10-USDA-725	2/4/2011	*	Validation	0.42	0.01	-0.17	-2.07
CS10-USDA-743	2/4/2011	*	Calibration	-0.1	0.02	0.57	0.26
CS10-USDA-765	2/4/2011	*	Calibration	-0.26	-0.04	-0.55	0.8
CS10-USDA-816	2/4/2011	*	Calibration	-0.1	-0.01	0.13	0.2

CS10-USDA-835	2/4/2011	*	Calibration	0.41	0	0	-0.53
CS10-USDA-862	2/4/2011	*	Calibration	0.09	-0.08	0.57	-0.75
CS10-USDA-880	2/4/2011	*	Calibration	0.43	-0.01	-0.62	1.18
CS10-WHTT-009	5/9/2011	((Tx114 (B73w)-B x CML343/Tx110 x Pop24)-B-B-B-4-B-B-B-B/CML78)-B-2-B-2-B/LH195	Calibration	-0.14	-0.03	-0.5	0.25
CS10-WHTT-012	5/9/2011	(CML379/CML311-B-1-B-B-B-B/Tx110)-B-1-B-2-B/LH287RR2	Validation	-0.02	0.02	-0.3	-0.31
CS10-WHTT-029	5/9/2011	(CML450-B/Tx110)-B-2-B-2-B/LH287RR2	Calibration	-0.14	0.02	-0.16	-0.58
CS10-WHTT-072	5/9/2011	W08-LH287-131	Calibration	-0.47	0.04	-0.14	0.15
CS10-WHTT-096	5/9/2011	(\CML450-B/Tx110)-B-1-B-1-B/LH195	Calibration	-0.15	0	-0.37	0.83
CS10-WHTT-114	5/9/2011	(CML379/CML311-B-1-B-B-B-B/Tx110)-B-1-B-2-B/LH195	Calibration	-0.22	0	0.16	-0.9
CS11-AMQY-120	12/7/2011	Tx 811 Q10 X CML 161 Q3	Calibration	-0.31	0.02	-0.19	-2.45
CS11-AMQY-163	12/7/2011	Tx 812 Q11 X LH195 Q6	Calibration	0.04	0.01	-0.15	-2.05
CS11-AMQY-202	12/7/2011	Tx 812 Q11 X Hallauer1 Q1	Validation	-0.52	-0.01	-0.16	-1.63
CS12-DYTL-101A	1/3/2016	AMATLCOHS71-1-1-2-1-1-1-BBBB-B-B-B	Validation	0.13	0.01	-0.28	1.35
CS12-DYTL-146A	1/3/2016	B64	Calibration	-0.03	-0.04	0.03	0.14
CS12-DYTL-221A	1/3/2016	Ki3	Calibration	0.13	0	0.3	2.53
CS12-DYTL-228A	1/3/2016	Pa91	Calibration	-0.28	0.01	-0.16	2.81
CS12-DYTL-234A	1/3/2016	Mt42	Calibration	0.22	0.03	0.01	4.17
CS12-DYTL-240A	1/3/2016	H99	Calibration	0.02	-0.01	-0.24	1.08
CS12-DYTL-242A	1/3/2016	CO255	Calibration	-0.09	-0.01	0.12	0.39
CS12-DYTL-258A	1/3/2016	NC368	Validation	-0.08	0	-0.17	-2.75
CS12-DYTL-294A	1/3/2016	I137TN	Calibration	-0.06	0.02	-0.22	-3.36
CS12-DYTL-334A	1/3/2016	A682	Validation	0.22	0	-0.33	-0.09
CS12-DYTL-376A	1/3/2016	CML158Q	Calibration	-0.45	0.03	0.33	-4.9
CS12-DYTL-437A	1/3/2016	Mp07:153	Calibration	0.01	0.04	0.15	-1.96
CS12-DYTL-596A	1/3/2016	P39Goodman-Buckler	Calibration	-0.45	-0.01	-0.23	1.22

CS12-DYTL-641A	1/3/2016	38-11	Calibration	-0.06	0.01	-0.15	-1.05
CS12-DYTL-671A		Tzi10	Calibration	0.04	-0.09	-0.08	-0.05
CS12-DYTL-680A	1/3/2016	CO255	Validation	0.02	0.02	0.25	4.9
CS12-DYTL-754A	1/3/2016	Ki43	Calibration	0.14	0.01	0.74	4.52
CS12-DYTL-782A	1/3/2016	Ki3	Calibration	-0.03	-0.02	-0.06	5.51
CS12-DYTL-783A	1/3/2016	GT112	Calibration	-0.28	0.06	-0.05	5.71
CS12-DYTL-794A	1/3/2016	[MBRC6BcG395-1-B-#-2-2-B-B-B-B-B/CML312SR]-1-1	Calibration	-0.17	0.04	0.3	6.97
CS12-DYTL-910A	1/3/2016	CML77	Calibration	0.02	0.04	0.67	-0.1
CS12-LIYT-111A	1/3/2016	CMV3	Calibration	-0.11	0.01	-0.11	2.03
CS12-LIYT-125A	1/3/2016	H95	Validation	0	-0.01	-0.52	1.81
CS12-LIYT-162A	1/3/2016	Oh603	Calibration	-0.19	0.02	0.04	-0.5
CS12-LIYT-185A	1/3/2016	NC264	Validation	-0.32	-0.01	-0.07	2.18
CS12-LIYT-305A	1/3/2016	Mo47	Calibration	-0.14	0.02	-0.24	2.96
CS12-LIYT-532A	1/3/2016	NC322	Calibration	-0.17	0	-0.31	4.11
CS12-LIYT-547A	1/3/2016	Ki43	Calibration	0.17	-0.02	-0.02	1.17
CS12-LIYT-745A	1/3/2016	NC358	Calibration	-0.11	-0.02	0.09	-0.03
CS12-LIYT-752A	1/3/2016	GT112	Calibration	-0.27	-0.01	0.04	-1.34
CS12-RJW-034-1	5/13/2014	*	Calibration	0.19	-0.04	0.25	-1.69
CS12-RJW-10-414	5/13/2014	*	Calibration	-0.03	0.01	-0.24	-2.51
CS12-RJW-10-428	5/13/2014	*	Calibration	0.24	0	0.28	2.8
CS12-RJW-10-435	5/13/2014	*	Calibration	0.23	0.06	0.53	-1.53
CS12-RJW-10-546	5/13/2014	*	Calibration	0.1	-0.04	-0.54	-1.79
CS12-RJW-10-573	5/13/2014	*	Calibration	-0.62	0.04	-0.17	-2.92
CS12-RJW-10-647	5/13/2014	*	Validation	0.23	0.01	-0.52	-1.25
CS12-RJW-161-1	5/13/2014	*	Calibration	-0.18	0.02	-1.01	0.18
CS12-RJW-165-1	5/13/2014	*	Validation	0.15	0.01	0.27	0.36

CS12-RJW-250-1	5/13/2014	*	Calibration	-0.46	0.02	0.22	0.94
CS12-RJW-261-1	5/13/2014	*	Calibration	0.27	0.01	0.87	-1.02
CS12-RJW-349-1	5/13/2014	*	Calibration	0.43	-0.01	-0.47	-0.49
CS12-RJW-390-1	5/13/2014	*	Calibration	0.28	0.03	0.47	0.64
CS12-RJW-527-1	5/13/2014	*	Calibration	-0.09	0	-0.16	-1.43
CS12-RJW-8-40	5/13/2014	*	Validation	0.35	-0.01	-0.16	-0.35
CS12-RJW-8-51	5/13/2014	*	Calibration	0.17	0.02	0.12	-0.63
CS12-RJW-9-220	5/13/2014	*	Calibration	0.9	0.04	-0.16	-1.44
CS12-RJW-9-236	5/13/2014	*	Calibration	0.04	0.01	0.35	-0.94
CS13-1AF2-026	5/13/2014	GT603 -B/((Tx740) ; LAMA2002-12-1-B-B-B)-B10	Calibration	0.22	-0.02	0.17	-1.32
CS13-1AF2-032	5/13/2014	((Tx740) ; LAMA2002-12-1-B-B-B)-B10/Mp718	Calibration	-0.17	0.01	0.38	0.85
CS13-1AF2-062	5/13/2014	BH8928VTTP	Validation	-0.15	-0.03	-0.25	0.6
CS13-B-398	5/13/2014	*	Calibration	0.43	0.01	0.24	7.56
CS13-DOTH-098-B9	5/13/2014	(((RedHybridEar-B-1-2-2-1-B)//(Lfy2361-B/Tx114(B73w)-BDarkblue-B)Tx114/Lfy2304-B-B-B-1-2-B-B-B-2-B)///((RedEar5-2-4-1-1-2)/(Ethiopia15-B-5-1-B-B2-B-1-B-B)))-5#-1-B4-B4-B9	Calibration	0.63	0.01	0.14	0.99
CS13-DOTH-102-B2	5/13/2014	4 way cross progeny (Tx772, B73 Oleic, Tx903, Tx906)	Validation	-0.04	-0.01	0.14	-0.44
CS13-EOTH-21-B10	5/13/2014	87916'	Calibration	0.12	0.01	-0.15	-0.02
CS13-EOTH-52-B4	5/13/2014	(((PB80/(Ethiopia15-B-5-1-B-B2-B-1-B-B)-B4#-2)//(PB80/(Ethiopia15-B-5-1-B-B2-B-1-B-B)-B4#-1))-B5//HopiBlue)-B7-B4	Calibration	-0.12	0.02	-0.12	-0.33
CS13-INC-020-B18	5/13/2014	LAMA2002-58-3-B-B-B-B-B-B-1-B19-B18	Calibration	0.43	0.02	0.16	-0.27
CS13-LINC-036-B11		Tx772	Calibration	-3.27	-0.07	0.16	4.78
CS13-LINC-040-B22		Tx903 - (Lfy2361-B/Tx114(B73w)-B Dark blue-B)Tx114/Lfy2304-B-B-B-1-3-B-B-B-3-B-B-B22	Calibration	-0.25	-0.01	0.24	1.46
CS13-OTH-055-X-OTH-049	5/13/2014	((((LH123HT/(RedEar5-2-4-1-1-2))-1-B4-1#)//LH123HT)-B2///LH123HT)	Calibration	0.28	0	0	2.94

CS13-OTH-114-B2	5/13/2014	(PHG84/WC1082)-B3-6-B5	Calibration	0.25	0	0.27	0.7
CS13-SCAH-003	5/13/2014	TR6282/(((B104/NC300)x(CML 415/B104))-4-2-B-B-B/LAMA2002-22-3-B-B1)-B-B-B-B-B	Calibration	-0.06	0.01	-0.16	0.29
CS13-SCAH-016	5/13/2014	Tx772-B-B-B-B-B-1/LH195	Calibration	0.11	-0.02	-0.26	-0.54
CS13-SCAH-029	5/13/2014	Tx772-B-B-B-B-B-1/LH195	Calibration	0.02	-0.03	-0.49	-0.21
CS13-SERAT-010-OP10	5/13/2014	Tx-WX13-6	Validation	0	0.01	-0.14	0.48
CS13-SERAT-022-SIB	5/13/2014	GT-A2R x Mo17	Calibration	-0.1	0	-0.39	-0.18
CS13-SERAT-052-SIB	5/13/2014	GT-A2R x B73	Calibration	0.22	0	0.1	2.22
CS13-SERAT-053-OP10	5/13/2014	GEMS 0005-2-1B X Hi27bs	Calibration	0.27	-0.05	-0.93	0.82
CS13-SERAT-060-OP9	5/13/2014	Tx-WX13-7	Validation	0.25	-0.02	-0.11	1.79
CS13-SERAT-071-SIB	5/13/2014	SS4 X (((B104/NC300)x(CML 415/B104))-4-2-B-B-B/LAMA2002-22-3-B-B1)-B-B-B-B-B	Calibration	0.4	0	-0.32	1.53
CS13-SERAT-080-OP10	5/13/2014	BH8740VTTP	Calibration	0.18	-0.03	0.33	1.17
CS13-SERAT-093-SIB	5/13/2014	Tx-WX13-5	Calibration	-0.03	-0.02	0.01	3.09
CS14-SERAT-005	2/18/2015	((Tx740/Mp715)//(Tx772/Mp313))-#/((Tx772/Mp715)//(Tx740/Mp313E))-#	Calibration	0.16	-0.01	-0.13	1.5
CS14-SERAT-51-OP10	2/18/2015	Mp13:9025 x Mp13:9026	Calibration	0.04	0.01	-0.07	0.44
CS14-SERAT-70-OP9	2/18/2015	((Tx740/Mp715)//(Tx772/Mp313))-#/((Tx772/Mp715)//(Tx740/Mp313E))-#	Calibration	0.17	-0.02	-0.35	1.34
CS14-SERAT-104	2/18/2015	SS1\X\(LAMA2002-35-2-B-B-B-B/CG44)-1-3-B-B14-B10	Calibration	-0.03	0	-0.56	-0.38
CS14-SERAT-106	2/18/2015	GRACE E-5 (E-1) x DK888	Calibration	-0.22	0.01	-0.18	-0.93
CS14-SERAT-108	2/18/2015	Tx-WX-8	Calibration	0.15	0	-0.18	-2.9
CS14-SERAT-109	2/18/2015	*	Calibration	0.28	-0.01	0.05	-0.23
CS14-SERAT-21	2/18/2015	Tx-WX-9	Calibration	-0.01	0.01	-0.35	1.72
CS14-SERAT-46	2/18/2015	Tx-WX-8	Calibration	-0.23	-0.02	-0.3	-0.7
CS14-SERAT-75	2/18/2015	Mp13:9035 x Mp13:9036	Calibration	-0.06	-0.03	-0.93	0.05
CS14-SERAT-81	2/18/2015	Hi63 x NC466	Calibration	0.07	0.01	-0.35	-2.29
CS15-NILAS-8047	1/3/2016	*	Calibration	0.08	0.02	0.66	-0.32

CS15-NILAS-8049	1/3/2016	*	Calibration	0.32	0.04	0.14	-1.71
CS15-NILAS-8054	1/3/2016	*	Calibration	0.11	0.04	0.17	-1.42
CS15-NILAS-8081	1/3/2016	*	Calibration	0.16	-0.02	0.12	-3.08
CS15-NILAS-8145	1/3/2016	*	Calibration	0.43	0	-0.18	-0.56
CS15-NILAS-8157	1/3/2016	*	Calibration	0.53	0	-0.16	1.23
CS15-NILAS-8168	1/3/2016	*	Calibration	0.17	-0.04	-0.11	2.76
CS15-NILAS-8323	1/3/2016	*	Validation	0.08	0.04	0.34	-1.21
CS15-NILAS-8368	1/3/2016	*	Calibration	0.32	-0.04	-0.42	2.37
CS15-NILAS-8900	1/3/2016	*	Calibration	0.27	-0.01	0.09	-3.95
IA11-AMQY-113	12/7/2011	*	Calibration	-0.44	0.02	0.49	1.09
IA11-AMQY-128	12/7/2011	*	Calibration	0.27	-0.01	0.2	1.64
IA11-AMQY-145	12/7/2011	*	Calibration	0.12	-0.03	0.07	-0.94
IA11-AMQY-165	12/7/2011	*	Calibration	-0.35	-0.01	0.2	1.54
IA15-NILAS-1621	1/3/2016	*	Calibration	-0.37	-0.02	0.37	0.61
IA15-NILAS-1637	1/3/2016	*	Validation	-0.56	-0.05	0.19	-2.98
IA15-NILAS-1642	1/3/2016	*	Validation	-0.25	0	0.36	2.06
IA15-NILAS-1672	1/3/2016	*	Validation	-0.29	-0.03	0.37	1.06
IA15-NILAS-1961	1/3/2016	*	Calibration	-0.1	-0.04	-0.07	-1.26
IA15-NILAS-2075	1/3/2016	*	Calibration	0.11	-0.01	0.07	-1.34
IA15-NILAS-2100	1/3/2016	*	Validation	-0.21	-0.01	0.42	-0.81
IA15-NILAS-2108	1/3/2016	*	Calibration	0.18	0	-0.13	-3.72
IA15-NILAS-2584	1/3/2016	*	Calibration	0.14	0.01	0.34	-3.93
IA15-NILAS-2681	1/3/2016	*	Validation	-0.05	0.02	0.29	-0.49
IA15-NILAS-2725	1/3/2016	*	Validation	-0.17	0.01	0.26	-0.94
IA15-NILAS-2944	1/3/2016	*	Calibration	-0.2	0	0.6	-0.28
IA15-NILAS-2962	1/3/2016	*	Calibration	0.06	0	0.2	-0.62
IA15-NILAS-2980	1/3/2016	*	Validation	-0.02	0	0.12	-1.36

IA15-NILAS-2992	1/3/2016	*	Validation	-0.05	0.02	0.13	-3.83
IA15-NILAS-3043	1/3/2016	*	Calibration	0.02	-0.04	-0.21	-4.34
IA15-NILAS-3046	1/3/2016	*	Calibration	-0.07	-0.03	0.25	-4.16
IA15-NILAS-3078	1/3/2016	*	Calibration	-0.2	-0.06	-0.03	-1.83
IA15-NILAS-3122	1/3/2016	*	Calibration	-0.4	0	0.14	1.34
TH10-TAC-101A	2/4/2011	TAC-5	Calibration	-0.28	0	-0.6	1.16
TH10-TAC-145A	2/4/2011	TAC-8	Calibration	-0.07	0.01	0.1	-1.41
TH10-TAC-221A	2/4/2011	TAC-5	Validation	-0.04	0.03	-0.31	-0.9
TH10-TAC-319A	2/4/2011	TAC-8	Validation	0.1	0	-0.01	0.04
TH10-TAC-336A	2/4/2011	TAC-5	Calibration	0.1	0.02	0.16	0.73
TH10-TAC-401A	2/4/2011	TAC-8	Calibration	0.19	-0.03	-0.35	0.86
WE10-ARG1-25	5/9/2011	ArgnetineFlintyComposite-C(1)-14-B-B/LH287RR2	Validation	-0.19	-0.06	0.63	-0.87
WE10-ARG1-48	5/9/2011	ArgnetineFlintyComposite-C(1)-3-B-B/LH287RR2	Calibration	-0.05	0.03	0.26	-0.17
WE10-QPMX-025		((CML269/Tx110)/(CML311/Tx110)-1-B-B-B-B/DTPWC8F31-1-1-2-2-BBBB-B)-B-B-3-2-B-B-B/X_WHTQPMTSTR	Validation	-0.15	-0.02	-0.32	-1.75
WE10-QPMX-033	5/9/2011	Pop.69TempladoAmarilloQPM-B-B-B2-12-B-B-B-B-B-B-B-1/X_WHTQPMTSTR	Calibration	-0.25	0.01	-0.3	-0.6
WE10-QPMX-035	5/9/2011	(B97xCML326-B/Tx770xA645)-2-2-B-B-B-B-B-B-B//X_WHTQPMTSTR	Calibration	-0.09	0.03	-0.31	-0.9
WE10-QPMX-094	5/9/2011	P31G66(2007)	Calibration	0.48	-0.04	-0.58	-0.7
WE10-QPMX-132	5/9/2011	(LAMA2002-12-1-B/(CML325/B104)-B-1-B-B-B-B)-B-B2-4-2-B-B-B//X_WHTQPMTSTR	Calibration	0.09	-0.01	-0.27	-0.51
WE10-QPMX-196	5/9/2011	Tx804//X_WHTQPMTSTR	Calibration	-0.14	0	-0.73	0.34
WE10-WHTT-011	5/9/2011	((Tx114(B73w)-BxCML343/Tx110xPop24)-B-B-B-4-B-B-B-B/CML78)-B-2-B-1-B/LH287RR2	Validation	-0.01	0.03	0.3	-1.18
WE10-WHTT-091	5/9/2011	((CML373/FR825)/(CML269/Tx110)-1-B-B-B-B/CML269/TX114-B-B-B-1-1-B-B-B)-B-1-B-3-B/LH195	Calibration	0.18	-0.01	0.26	-0.49

WE10-WHTT-105	5/9/2011	((CML373/FR825)/(CML269/Tx110)-1-B-B-B-B/CML269/TX114-B-B-B-1-1-B-B-B-B-B)-B-1-B-3-B/LH195	Validation	-0.1	-0.02	-0.6	0.37
WE11-AMQY-129			Calibration	-0.02	0	-0.12	-1.57
WE11-AMQY-150			Calibration	-0.65	-0.02	0.08	-0.31
WE11-AMQY-159	12/7/2011	Tx 812 Q11 X LH195 Q6	Calibration	0.45	-0.01	-0.28	-1.05
WE11-AMQY-215	12/7/2011	DKC68-06	Calibration	0.11	-0.01	-0.39	1.06
WE11-AMQY-267	12/7/2011	Tx 810 Q9 X CML 176 Q5	Calibration	0.14	0	0.31	-1.12
WE13-SCAH-13-OP46	5/13/2014	TR6282/(((B104/NC300)x(CML 415/B104))-4-2-B-B-B/LAMA2002-22-3-B-B1)-B-B-B-B-B	Validation	0.16	0.06	0.36	3.41
WE13-SCAH-29-OP50	5/13/2014	Tx772-B-B-B-B-B-1/LH195	Calibration	-0.11	-0.01	0	-1.64
WE13-SCAH-3-OP49	5/13/2014	TR6282/(((B104/NC300)x(CML 415/B104))-4-2-B-B-B/LAMA2002-22-3-B-B1)-B-B-B-B-B	Calibration	0.05	0.03	-0.02	1.31
WE13-SCAH-30-OP51	5/13/2014	TR6282/(((B104/NC300)x(CML 415/B104))-4-2-B-B-B/LAMA2002-22-3-B-B1)-B-B-B-B-B	Calibration	0.45	-0.02	0.09	2.66

APPENDIX F

CALIBRATION MODELS WITH SUMMARY STATISTICS FOR WHOLE KERNEL MAIZE PHOSPHORUS

Calibration model	r	PLS factors	PI	RMSEC
PLS_MSC_1D_SG_3-1	0.92	6	56.3	0.02
PLS_MSC_1D_SG_3-3	0.92	6	56.3	0.02
PLS_MSC_1D_SG_3-5	0.92	6	56.3	0.02
PLS_MSC_1D_SG_5-1	0.85	6	61.4	0.03
PLS_MSC_1D_SG_5-3	0.95	7	56.6	0.02
PLS_MSC_1D_SG_5-5	0.95	7	56.6	0.02
PLS_MSC_1D_SG_7-1	0.80	6	64	0.03
PLS_MSC_1D_SG_7-3	0.91	6	55.8	0.02
PLS_MSC_1D_SG_7-5	0.96	7	56.3	0.02
PLS_MSC_1D_SG_11-1	0.77	6	64.4	0.03
PLS_MSC_1D_SG_11-3	0.83	6	63.8	0.03
PLS_MSC_1D_SG_11-5	0.78	4	53.6	0.03
PLS_MSC_1D_SG_19-1	0.71	6	61.6	0.04
PLS_MSC_1D_SG_19-3	0.71	5	62.6	0.04
PLS_MSC_1D_SG_19-5	0.81	6	64.7	0.03
PLS_CON_1D_SG_3-1	0.94	7	57.3	0.02
PLS_CON_1D_SG_3-3	0.94	7	57.3	0.02
PLS_CON_1D_SG_3-5	0.94	7	57.3	0.02
PLS_CON_1D_SG_5-1	0.88	7	64.3	0.03
PLS_CON_1D_SG_5-3	0.97	8	55.5	0.02
PLS_CON_1D_SG_5-5	0.97	8	55.5	0.01
PLS_CON_1D_SG_7-1	0.83	7	67.1	0.03
PLS_CON_1D_SG_7-3	0.93	7	57.9	0.02
PLS_CON_1D_SG_7-5	0.97	8	54.9	0.01
PLS_CON_1D_SG_11-1	0.76	7	65.5	0.04

PLS_CON_1D_SG_11-3	0.92	9	57.3	0.02
PLS_CON_1D_SG_11-5	0.92	7	58.2	0.02
PLS_CON_1D_SG_19-1	0.73	7	65.2	0.04
PLS_CON_1D_SG_19-3	0.79	8	62.7	0.03
PLS_CON_1D_SG_19-5	0.83	7	69.5	0.03
PLS_CON_2D_SG_3-1	0.85	3	43.7	0.03
PLS_CON_2D_SG_3-3	0.85	3	43.7	0.03
PLS_CON_2D_SG_3-5	0.85	3	43.7	0.03
PLS_CON_2D_SG_5-1	0.86	3	41.9	0.03
PLS_CON_2D_SG_5-3	0.86	3	41.9	0.03
PLS_CON_2D_SG_5-5	0.85	3	43.9	0.03
PLS_CON_2D_SG_7-1	0.95	4	50.5	0.02
PLS_CON_2D_SG_7-3	0.95	4	50.5	0.00
PLS_CON_2D_SG_7-5	0.85	3	40.7	0.03
PLS_CON_2D_SG_11-1	0.94	5	64.2	0.02
PLS_CON_2D_SG_11-3	0.94	5	64.2	0.02
PLS_CON_2D_SG_11-5	0.98	5	52	0.01
PLS_CON_2D_SG_19-1	0.93	8	64.9	0.02
PLS_CON_2D_SG_19-3	0.93	8	64.9	0.02
PLS_CON_2D_SG_19-5	0.95	6	55	0.02
PLS_MSC_2D_SG_3-1	0.87	3	42.4	0.03
PLS_MSC_2D_SG_3-3	0.87	3	42.4	0.03
PLS_MSC_2D_SG_3-5	0.87	3	42.4	0.03
PLS_MSC_2D_SG_7-1	0.95	4	50.9	0.02
PLS_MSC_2D_SG_7-3	0.95	4	50.9	0.02
PLS_MSC_2D_SG_7-5	0.87	3	40.5	0.03
PLS_MSC_2D_SG_11-1	0.97	6	63.4	0.01
PLS_MSC_2D_SG_11-3	0.97	6	63.4	0.01
PLS_MSC_2D_SG_11-5	0.955	4	49	0.016

APPENDIX G

CALIBRATION MODELS WITH SUMMARY STATISTICS FOR WHOLE KERNEL MAIZE CRUDE PROTEIN

Calibration model	r	PLS factors	PI	RMSEC
PLS_MSC_1D_SG_3-1	0.97	7	57.1	0.42
PLS_MSC_1D_SG_3-3	0.97	7	57.1	0.42
PLS_MSC_1D_SG_3-5	0.97	7	57.1	0.42
PLS_MSC_1D_SG_5-1	0.98	9	62.9	0.36
PLS_MSC_1D_SG_5-3	0.98	8	54.7	0.29
PLS_MSC_1D_SG_5-5	0.98	8	54.7	0.29
PLS_MSC_1D_SG_7-1	0.96	9	70.5	0.44
PLS_MSC_1D_SG_7-3	0.98	8	58.5	0.32
PLS_MSC_1D_SG_7-5	0.99	8	54.3	0.27
PLS_MSC_1D_SG_11-1	0.92	8	67.9	0.63
PLS_MSC_1D_SG_11-3	0.98	10	67.2	0.31
PLS_MSC_1D_SG_11-5	0.98	8	59.2	0.34
PLS_MSC_1D_SG_19-1	0.92	9	75.7	0.64
PLS_MSC_1D_SG_19-3	0.91	8	67.8	0.66
PLS_MSC_1D_SG_19-5	0.95	9	69	0.49
PLS_CON_1D_SG_3-1	0.97	8	59.7	0.38
PLS_CON_1D_SG_3-3	0.97	8	59.7	0.38
PLS_CON_1D_SG_3-5	0.97	8	59.7	0.38
PLS_CON_1D_SG_5-1	0.98	10	61.4	0.33
PLS_CON_1D_SG_5-3	0.98	8	56.1	0.36
PLS_CON_1D_SG_5-5	0.98	8	56.1	0.36
PLS_CON_1D_SG_7-1	0.97	10	65.1	0.39
PLS_CON_1D_SG_7-3	0.99	10	56	0.22
PLS_CON_1D_SG_7-5	0.98	8	54.8	0.32

PLS_CON_1D_SG_11-1	0.91	9	70.7	0.66
PLS_CON_1D_SG_11-3	0.97	10	61.9	0.38
PLS_CON_1D_SG_11-5	0.98	9	60.5	0.34
PLS_CON_1D_SG_19-1	0.90	9	69.1	0.70
PLS_CON_1D_SG_19-3	0.92	9	70.9	0.64
PLS_CON_1D_SG_19-5	0.96	10	64.7	0.44
PLS_CON_2D_SG_3-1	0.34	1	34.6	1.52
PLS_CON_2D_SG_3-3	0.34	1	34.6	1.52
PLS_CON_2D_SG_3-5	0.34	1	34.6	1.52
PLS_CON_2D_SG_5-1	0.34	1	35	1.52
PLS_CON_2D_SG_5-3	0.34	1	35	1.52
PLS_CON_2D_SG_5-5	0.34	1	34.6	1.52
PLS_CON_2D_SG_7-1	0.86	3	37.4	0.82
PLS_CON_2D_SG_7-3	0.86	3	37.4	0.82
PLS_CON_2D_SG_7-5	0.33	1	34.9	1.52
PLS_CON_2D_SG_11-1	0.99	7	37	0.26
PLS_CON_2D_SG_11-3	0.99	7	37	0.26
PLS_CON_2D_SG_11-5	0.87	3	34.5	0.79
PLS_CON_2D_SG_19-1	0.99	10	68.1	0.26
PLS_CON_2D_SG_19-3	0.99	10	68.1	0.26
PLS_CON_2D_SG_19-5	0.99	8	41.4	0.22
PLS_MSC_2D_SG_3-1	0.28	1	35.4	1.55
PLS_MSC_2D_SG_3-3	0.28	1	35.4	1.55
PLS_MSC_2D_SG_3-5	0.28	1	35.4	1.55
PLS_MSC_2D_SG_7-1	0.86	3	34.9	0.83
PLS_MSC_2D_SG_7-3	0.86	3	34.9	0.83
PLS_MSC_2D_SG_7-5	0.28	1	35.5	1.55
PLS_MSC_2D_SG_11-1	0.99	7	38.1	0.20
PLS_MSC_2D_SG_11-3	0.99	7	38.1	0.20
PLS_MSC_2D_SG_11-5	0.86	3	32.5	0.83

APPENDIX H

CALIBRATION MODELS WITH SUMMARY STATISTICS FOR WHOLE KERNEL MAIZE FAT

Calibration model	r	PLS factors	PI	RMSEC
PLS_MSC_1D_SG_3-1	0.91	6	58.2	0.38
PLS_MSC_1D_SG_3-3	0.91	6	58.2	0.38
PLS_MSC_1D_SG_3-5	0.91	6	58.2	0.38
PLS_MSC_1D_SG_5-1	0.70	4	53.5	0.66
PLS_MSC_1D_SG_5-3	0.94	6	58	0.31
PLS_MSC_1D_SG_5-5	0.94	6	58	0.31
PLS_MSC_1D_SG_7-1	0.63	3	47.5	0.72
PLS_MSC_1D_SG_7-3	0.74	4	54.8	0.62
PLS_MSC_1D_SG_7-5	0.87	5	52.3	0.46
PLS_MSC_1D_SG_11-1	0.62	3	53.1	0.73
PLS_MSC_1D_SG_11-3	0.70	4	51.3	0.66
PLS_MSC_1D_SG_11-5	0.73	4	55.2	0.63
PLS_MSC_1D_SG_19-1	0.57	3	52.5	0.76
PLS_MSC_1D_SG_19-3	0.61	3	51.9	0.73
PLS_MSC_1D_SG_19-5	0.74	5	54.6	0.63
PLS_CON_1D_SG_3-1	0.91	6	52.8	0.39
PLS_CON_1D_SG_3-3	0.91	6	52.8	0.39
PLS_CON_1D_SG_3-5	0.91	6	52.8	0.39
PLS_CON_1D_SG_5-1	0.72	5	54.6	0.64
PLS_CON_1D_SG_5-3	0.91	6	58.1	0.38
PLS_CON_1D_SG_5-5	0.91	6	58.1	0.38
PLS_CON_1D_SG_7-1	0.62	3	48.3	0.72
PLS_CON_1D_SG_7-3	0.75	5	52.3	0.61
PLS_CON_1D_SG_7-5	0.85	5	38.6	0.49
PLS_CON_1D_SG_11-1	0.61	4	52.9	0.73
PLS_CON_1D_SG_11-3	0.87	7	64.5	0.45

PLS_CON_1D_SG_11-5	0.75	5	53.2	0.61
PLS_CON_1D_SG_19-1	0.58	4	49.7	0.75
PLS_CON_1D_SG_19-3	0.62	4	53.2	0.73
PLS_CON_1D_SG_19-5	0.63	3	47.9	0.72
PLS_CON_2D_SG_3-1	0.97	4	41	0.23
PLS_CON_2D_SG_3-3	0.97	4	41	0.23
PLS_CON_2D_SG_3-5	0.97	4	41	0.23
PLS_CON_2D_SG_5-1	0.99	5	43.7	0.14
PLS_CON_2D_SG_5-3	0.99	5	43.7	0.14
PLS_CON_2D_SG_5-5	0.84	3	35.3	0.50
PLS_CON_2D_SG_7-1	1.00	6	48.6	0.08
PLS_CON_2D_SG_7-3	1.00	6	48.6	0.08
PLS_CON_2D_SG_7-5	0.85	3	38.6	0.49
PLS_CON_2D_SG_11-1	0.86	3	61.7	0.47
PLS_CON_2D_SG_11-3	0.86	3	61.7	0.47
PLS_CON_2D_SG_11-5	0.95	3	45	0.29
PLS_CON_2D_SG_19-1	0.83	5	55.3	0.51
PLS_CON_2D_SG_19-3	0.83	5	55.3	0.51
PLS_CON_2D_SG_19-5	0.83	3	61.9	0.51
PLS_MSC_2D_SG_3-1	0.82	3	36.9	0.53
PLS_MSC_2D_SG_3-3	0.82	3	36.9	0.53
PLS_MSC_2D_SG_3-5	0.82	3	36.9	0.53
PLS_MSC_2D_SG_7-1	0.83	3	40.1	0.52
PLS_MSC_2D_SG_7-3	0.83	3	40.1	0.52
PLS_MSC_2D_SG_7-5	0.82	3	36.3	0.52
PLS_MSC_2D_SG_11-1	0.88	4	64.7	0.45
PLS_MSC_2D_SG_11-3	0.88	4	64.7	0.45
PLS_MSC_2D_SG_11-5	0.83	3	40.4	0.51

APPENDIX I

CALIBRATION MODELS WITH SUMMARY STATISTICS FOR WHOLE KERNEL MAIZE STARCH

Calibration model	r	PLS factors	PI	RMSEC	
PLS_MSC_1D_SG_3-1	0.96	7	27.4	0.86	
PLS_MSC_1D_SG_3-3	0.96	7	27.4	0.86	
PLS_MSC_1D_SG_3-5	0.96	7	27.4	0.86	
PLS_MSC_1D_SG_5-1	0.92	7	29.8	1.19	
PLS_MSC_1D_SG_5-3	0.97	7	27.6	0.73	
PLS_MSC_1D_SG_5-5	0.97	7	27.6	0.73	
PLS_MSC_1D_SG_7-1	0.89	7	31.4	1.37	
PLS_MSC_1D_SG_7-3	0.98	8	27.1	0.57	
PLS_MSC_1D_SG_7-5	0.97	7	25.5	0.71	
PLS_MSC_1D_SG_11-1	0.82	5	33.1	1.74	
PLS_MSC_1D_SG_11-3	0.90	7	30.3	1.31	
PLS_MSC_1D_SG_11-5	0.93	6	29.3	1.12	
PLS_MSC_1D_SG_19-1	0.79	6	30.6	1.83	
PLS_MSC_1D_SG_19-3	0.82	6	31.5	1.73	
PLS_MSC_1D_SG_19-5	0.89	7	31.9	1.39	
PLS_CON_1D_SG_3-1	0.97	8	28.5	0.69	
PLS_CON_1D_SG_3-3	0.97	8	28.5	0.69	
PLS_CON_1D_SG_3-5	0.97	8	28.5	0.69	
PLS_CON_1D_SG_5-1	0.92	7	38.2	1.21	
PLS_CON_1D_SG_5-3	0.98	8	26.6	0.60	
PLS_CON_1D_SG_5-5	0.98	8	26.6	0.60	
PLS_CON_1D_SG_7-1	0.88	7	37.3	1.43	
PLS_CON_1D_SG_7-3	0.97	8	30.7	0.76	

PLS_CON_1D_SG_7-5	0.99	9	24.5	0.38
PLS_CON_1D_SG_11-1	0.81	6	32.3	1.77
PLS_CON_1D_SG_11-3	0.92	8	37.3	1.17
PLS_CON_1D_SG_11-5	0.96	8	30.1	0.81
PLS_CON_1D_SG_19-1	0.80	7	35.9	1.80
PLS_CON_1D_SG_19-3	0.82	6	33.1	1.74
PLS_CON_1D_SG_19-5	0.88	7	37	1.14
PLS_CON_2D_SG_3-1	0.97	4	-8.6	0.79
PLS_CON_2D_SG_3-3	0.97	4	-8.6	0.79
PLS_CON_2D_SG_3-5	0.97	4	-8.6	0.79
PLS_CON_2D_SG_5-1	0.96	4	-2.3	0.86
PLS_CON_2D_SG_5-3	0.96	4	-2.3	0.86
PLS_CON_2D_SG_5-5	0.97	4	-9.4	0.79
PLS_CON_2D_SG_7-1	0.96	4	11.4	0.89
PLS_CON_2D_SG_7-3	0.96	4	11.4	0.89
PLS_CON_2D_SG_7-5	0.96	4	-9.4	0.86
PLS_CON_2D_SG_11-1	0.96	5	23.8	0.89
PLS_CON_2D_SG_11-3	0.96	5	23.8	0.89
PLS_CON_2D_SG_11-5	0.95	4	8	0.91
PLS_CON_2D_SG_19-1	0.95	8	38.2	0.97
PLS_CON_2D_SG_19-3	0.95	8	38.2	0.97
PLS_CON_2D_SG_19-5	0.94	5	27.4	1.02
PLS_MSC_2D_SG_3-1	0.96	4	-8.4	0.85
PLS_MSC_2D_SG_3-3	0.96	4	-8.4	0.85
PLS_MSC_2D_SG_3-5	0.96	4	-8.4	0.85
PLS_MSC_2D_SG_7-1	0.98	5	3.6	0.67
PLS_MSC_2D_SG_7-3	0.98	5	3.6	0.67
PLS_MSC_2D_SG_7-5	0.95	4	-10.6	0.93
PLS_MSC_2D_SG_11-1	0.95	5	25.1	0.94
PLS_MSC_2D_SG_11-3	0.95	5	25.1	0.94

PLS_MSC_2D_SG_11-5	0.98	5	-1.3	0.64

APPENDIX J

CALIBRATION MODELS WITH SUMMARY STATISTICS FOR UDY GROUND MAIZE PHOSPHORUS

Calibration model	r	PLS factors	PI	RMSEC
PLS_MSC_1D_SG_5-1	0.91	8	62.7	0.02
PLS_MSC_1D_SG_11-1	0.86	8	60.3	0.03
PLS_MSC_1D_SG_19-1	0.89	10	64.2	0.03
PLS_MSC_1D_SG_19-5	0.88	8	61.2	0.03
PLS_CON_1D_SG_7-1	0.90	9	61.7	0.03
PLS_CON_1D_SG_11-5	0.79	5	55.4	0.03
PLS_MSC_1D_ND_1-11	0.92	10	65.5	0.02
PLS_MSC_1D_ND_3-7	0.92	10	65.3	0.02
PLS_MSC_1D_ND_7-1	0.91	10	64.8	0.02
PLS_CON_1D_ND_1-7	0.86	8	63.1	0.03
PLS_CON_1D_ND_7-1	0.90	10	62	0.03
PLS_CON_1D_ND_19-1	0.88	10	67	0.03
PLS_CON_1D_ND_19-5	0.87	10	66.4	0.03
PLS_CON_2D_ND_1-3	0.88	5	56.2	0.03
PLS_CON_2D_ND_7-1	0.78	5	54.2	0.04
PLS_CON_2D_ND_11-5	0.80	7	59	0.03
PLS_CON_2D_ND_19-5	0.88	10	65	0.03
PLS_MSC_2D_ND_19-5	0.85	9	63.1	0.03

APPENDIX K

CALIBRATION MODELS WITH SUMMARY STATISTICS FOR UDY GROUND MAIZE CRUDE PROTEIN

Calibration model	r	PLS factors	PI	RMSEC
PLS_MSC_1D_SG_5-1	0.97	7	84.1	0.39
PLS_MSC_1D_SG_11-1	0.98	8	84.7	0.39
PLS_MSC_1D_SG_19-5	0.98	8	83.7	0.37
PLS_CON_1D_SG_7-1	0.98	8	86.6	0.38
PLS_CON_1D_SG_7-3	0.99	10	85.6	0.21
PLS_CON_1D_SG_11-5	0.99	10	85.6	0.22
PLS_CON_1D_SG_19-3	0.98	8	87	0.38
PLS_MSC_1D_ND_7-1	0.97	8	85	0.40
PLS_CON_1D_ND_1-7	0.98	8	87.2	0.38
PLS_CON_1D_ND_7-1	0.97	8	86.2	0.40
PLS_CON_1D_ND_19-5	0.97	7	85.5	0.43
PLS_CON_2D_ND_1-3	0.98	8	66.6	0.32
PLS_CON_2D_ND_7-1	0.97	8	82.6	0.40
PLS_CON_2D_ND_11-5	0.97	9	85	0.43
PLS_CON_2D_ND_19-5	0.97	8	83.1	0.44
PLS_MSC_2D_ND_19-5	0.97	8	82.8	0.44

APPENDIX L

CALIBRATION MODELS WITH SUMMARY STATISTICS FOR UDY GROUND MAIZE FAT

Calibration model	r	PLS factors	PI	RMSEC
PLS_MSC_1D_SG_5-1	0.931	6	44.6	0.34
PLS_MSC_1D_SG_11-1	0.918	5	43.4	0.369
PLS_MSC_1D_SG_19-5	0.928	6	43.4	0.347
PLS_CON_1D_SG_5-3	0.974	7	49.6	0.209
PLS_CON_1D_SG_7-1	0.94	7	47.9	0.316
PLS_CON_1D_SG_7-5	0.975	7	50	0.205
PLS_CON_1D_SG_11-5	0.929	5	47.5	0.344
PLS_MSC_1D_ND_7-1	0.917	5	43.4	0.372
PLS_CON_1D_ND_1-5	0.942	7	48	0.312
PLS_CON_1D_ND_7-1	0.927	6	47.9	0.349
PLS_CON_1D_ND_19-5	0.926	7	46	0.351
PLS_CON_2D_ND_1-3	0.926	5	40.4	0.352
PLS_CON_2D_ND_1-11	0.941	6	49.2	0.313
PLS_CON_2D_ND_7-1	0.931	5	48	0.338
PLS_CON_2D_ND_11-5	0.919	4	46.2	0.367
PLS_CON_2D_ND_19-5	0.927	6	48.1	0.349
PLS_MSC_2D_ND_19-5	0.917	5	44.4	0.371

APPENDIX M

CALIBRATION MODELS WITH SUMMARY STATISTICS FOR UDY GROUND MAIZE STARCH

Calibration model	r	PLS factors	PI	RMSEC
PLS_MSC_1D_SG_5-1	0.915	7	22	1.28
PLS_MSC_1D_SG_11-1	0.885	6	21.5	1.47
PLS_MSC_1D_SG_19-5	0.904	7	22.3	1.36
PLS_CON_1D_SG_7-1	0.8464	5	17.5	1.69
PLS_CON_1D_SG_11-5	0.903	6	16.8	1.36
PLS_MSC_1D_ND_7-1	0.88	6	20.8	1.51
PLS_CON_1D_ND_7-1	0.907	8	29.4	1.33
PLS_CON_1D_ND_19-5	0.877	7	26	1.53
PLS_CON_2D_ND_1-3	0.973	8	14.7	0.736
PLS_CON_2D_ND_7-1	0.938	9	28.6	1.1
PLS_CON_2D_ND_11-5	0.91	9	29.7	1.32
PLS_CON_2D_ND_19-5	0.91	9	31.9	1.32
PLS_MSC_2D_ND_3-3	0.969	9	17.8	0.785
PLS_MSC_2D_ND_5-1	0.961	9	20.1	0.88
PLS_MSC_2D_ND_19-5	0.915	10	29.5	1.28

APPENDIX N

PHOSPHORUS MEANS SEPARATION FOR GENOTYPES SELECTED FROM FARFAN ET AL., 2015 FOR WESLACO

TRIAL

Genotype																																																										Mean
A441-5	A																																																								0.36
S2B73BC	A	B																																																							0.36
Ki3	A	B	C																																																						0.35
NC330	A	B	C	D																																																					0.35
U267Y	A	B	C	D	E																																																				0.35
IA2B2Goodman-Bu	A	B	C	D	E	F	G	H	I	J	K	L					Q	R		T	U																																					0.35
Ky21	A	B	C	D	E	F	G	H	I	J	K	L					Q	R		T	U																																					0.35
CML264	A	B	C	D	E	F	G	H	I	J	K	L					Q	R		T	U																																					0.35
NC316	A	B	C	D	E	F	G	H	I	J	K	L					Q	R		T	U																																					0.35
A634	A	B	C	D	E	F				J																																																0.35
NC222	A	B	C	D	E	F				J																																																0.35
Mp04:97	A	B	C	D	E					J																																																0.34
A214N	A	B	C	D	E	F				J																																																0.34
I29	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^	_	`	a	b	c	d	e	f		h		j	k		m						s							0.34
CML287	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]	^		`	a	b	c	d	e	f		h		j	k		m						s							0.34
Hi27Goodman-Buc	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R		T	U	V		X		Z									c																							0.34
A&M407-005	A	B	C	D	E	F	G			J							Q	R																																								0.34
D940Y	A	B	C	D	E	F	G	H	I	J							Q	R																																								0.34
SA24	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R		T	U	V		X		Z									c																							0.34
A654	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R		T	U	V		X		Z									c																							0.34
CI90C	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R		T	U	V		X		Z									c																							0.34
CML376	A	B	C	D	E	F	G			J							Q	R																																								0.34
AMATLCOHS71-1-1	A	B	C	D	E	F				J							Q	R																																								0.34
CML247	A	B	C	D	E	F	G	H		J							Q	R																																								0.34
B68	A	B	C	D	E	F				J								R																																								0.34
CML258	A	B	C	D	E	F	G	H		J							Q	R																																								0.34
CML432	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O		Q	R		T	U	V		X		Z																																0.34
Oh43E	A	B	C	D	E	F	G	H	I	J							Q	R																																								0.34
Ki14	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z	[	\	]				a		c					h		j																0.34
CML220																																							g					l		n	o	p	q	r		t	u	v	w	x	y	0.29
CML381																																							g					l		n	o	p	q	r		t	u	v	w	x	y	0.29

GA209																																						g					l		n	o	p	q	r		t	u	v	w	x	y	0.29
GT112																																														n		p	q	r				v	w	x	y	0.29
LaPostaSeqC7-F102-1-3-1-1-B-B-B																																														n		p	q	r				v	w	x	y	0.29
NC304																																						g					l		n	o	p	q	r		t	u	v	w	x	y	0.29
NC320																																														n		p	q	r				v	w	x	y	0.29
NC332																																														n		p	q	r				v	w	x	y	0.29
A556																																						g					l		n	o	p	q	r		t	u	v	w	x	y	0.29
ND246																																						g					l		n	o	p	q	r		t	u	v	w	x	y	0.29
NC348																													^	_	`		b		d		f	g		i		k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	0.29
NC264																																																p	q	r						x	y	0.29
H95																																														n		p	q	r				v	w	x	y	0.29
NC362																																														n		p	q	r				v	w	x	y	0.29
DE811																																						g					l		n		p	q	r			u	v	w	x	y	0.29
NC322																																																	q	r						x	y	0.29
W64A																																						g					l		n		p	q	r			u	v	w	x	y	0.29
NC370																																																	q	r				v		x	y	0.29
CMV3																																														n		p	q	r				v	w	x	y	0.29
W182B																																														n		p	q	r				v	w	x	y	0.29
NC302																																						g		i			l		n	o	p	q	r		t	u	v	w	x	y	0.29
T232																																						g		i			l		n	o	p	q	r		t	u	v	w	x	y	0.29
Mo46																																						g		i			l		n	o	p	q	r		t	u	v	w	x	y	0.29
NC334																																																		r						x	y	0.28
R168																													^	_	`		b		d	e	f	g		i		k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	0.28
CML103																													^	_	`		b		d	e	f	g		i		k	l	m	n	o	p	q	r	s	t	u	v	w	x	y	0.28
NC324																																														n		p	q	r			u	v	w	x	y	0.28
CO125																																																							w	x	y	0.28
LaPostaSeqC7-F64-2-4-1-1-B-B-B																																																							w	x	y	0.28
DK6305VT3P																																																								x		0.26